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THE RELATION BETWEEN NON-PROTEIN NITROGEN RETENTION AND PHENOLSULPHONEPHTHALEIN EXCRETION IN EXPERIMENTAL URANIUM NEPHRITIS *

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One of the important problems in connection with the study of nephritis is the determination of the ability of the kidneys to remove the metabolism products which normally find their way into the urine. Three different lines of investigation have been pursued in the study of this problem, namely, (a) urine analysis, the direct determination of urinary constituents, nitrogen, urea, chlorids, etc., (b) blood analysis, the determination of similar products in the blood, particularly the so-called uncoagulable nitrogen and the urea, (c) the determination of the ability of the kidney to eliminate certain foreign substances, such as potassium iodid or dyes, indigo-carmin, rosanilin, phenolsulphonephthalein, etc.

The first of these lines of investigation, urine analysis, has contributed very little, except in relation to the elimination of chlorids and water. To anyone familiar with the uncertainties and variations as well as the difficulties involved in nitrogen equilibrium experiments, this lack of definite results is by no means strange.

Investigation of the nitrogenous products retained in the blood in nephritis seemed from the beginning the most direct as well as the most promising point of attack, except for the great difficulties involved in determining the nitrogenous waste products in such a complex and highly nitrogenous fluid as blood. Accordingly, very little exact information is as yet available with regard to the relation between the accumulation

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of waste nitrogen in the blood and various stages and kinds of nephritis, although the literature on the subject is very voluminous.[1]

Studies of renal efficiency by means of various foreign substances, particularly dyes and indicators, have one great advantage in that they offer an almost unlimited scope for selection with reference to harmlessness, ease of estimation and the readiness with which the substances are eliminated by the kidneys. The chief drawback attached to this line of work lies in the interpretation of the results obtained, for it is quite conceivable that the rate with which the kidneys eliminate any one such foreign substance may have no definite relationship to the efficiency of the kidneys with reference to metabolism products. By far the most serviceable substance as yet introduced in this field is phenolsulphonephthalein.[2] In this paper will be found a review of the earlier literature on the subject. The numerous observations recorded by Rowntree and his associates on the speed with which phenolsulphonephthalein is eliminated by normal as well as by nephritic kidneys seem to show that the elimination of this substance when injected intramuscularly is indeed a valuable index to the general efficiency of the kidneys. The observations have been confirmed by several other investigators.[3]

The desirability of correlating the elimination of this substance with the actual retention in the blood of nitrogenous waste products is self-evident. Rowntree and Fitz[4] have already attempted such correlation, but so long as no reliable method existed for the determination of the urea and non-protein nitrogen in the blood the results obtained must necessarily remain obscure and uncertain. By means of the new methods of Folin and Denis[5] it is possible to determine with the necessary accuracy both the total non-protein nitrogen and urea in the blood.[6] These methods have the added advantage that only small quantities of blood (2 to 5 c.c.) are needed, thus making possible a continuous study of experimental nephritis in ordinary small laboratory animals, such as cats and rabbits.

For the work described in this paper we have used rabbits in whom acute nephritis was produced by means of a single dose of uranium

1. Strauss: die chronischen Nierenentzundungen, 1902; Widal: Compt. rend. Soc. de biol., 1904, p. 301; Obermayer and Popper: Ztschr. f. klin. Med., 1911, lxxii, 332; Hohlweg: Deutsch. Arch. f. klin. Med., 1911, civ, 216; Nellis Foster: THE ARCHIVES INT. MED., 1912, x, 414; Folin, Karsner and Denis: Jour. Exper. Med., 1912, xvi, 789.

2. Rowntree and Geraghty: Jour. Pharmacol. and Exper. Therap., 1910, i, 579.

3. Sehrl: Zentrlbl. f. Chir., 1912, No. 33, p. 1121; Deutsch. Wien. klin. Wchnschr., 1912, No. 32, p. 1217; Autenrieth and Funk: München. med. Wchnschr., 1912, No. 49, p. 2657; Erne: München. med. Wchnschr., 1913, No. 10, p. 510; Eisenbrey: Jour. Exper. Med., 1911, xiv, 462.

4. Rowntree and Fitz: THE ARCHIVES INT. MED., 1913, xi, 258.

5. Folin and Denis: Jour. Biol. Chem., 1912, xi, 527.

6. Folin and Dennis: Jour. Biol. Chem., 1913, xiv, 31.

nitrate (from 1.25 to 3 mg.) given subcutaneously. Two series of experiments were made. In the first series the animals were killed (under anesthesia) by bleeding from the carotid arteries. They were killed on consecutive days from one to ten days after the administration of the uranium nitrate in order to secure histological record of the pathological lesions in the kidney, and to determine their relation to the non-protein nitrogen in the blood and the urinary tests at different stages of the nephritis. In these experiments the blood was analyzed only on the day that the animals were killed. In the second series of experiments the animals were allowed to recover, and blood analyses as well as phenolsulphonephthalein tests were made periodically. The blood in these experiments was obtained from the veins of the ear. Because of the small quantity of blood taken each time (2 to 3 c.c.) no reduction in the hemoglobin of the animals was observed as a result of the frequent bleeding.

The rabbits were kept in cages of moderate size so that they could move around easily. They were fed with carrots and hay, and except for a few days at the height of the nephritis, each rabbit consumed about 100 grams of carrot per day. Just before the injection of the phenolsulphonephthalein solution (1 c.c. containing 6 mg. of the indicator) into the muscles of the thigh each rabbit was given 50 c.c. of water by means of a stomach tube. The animal was kept in a small cage over a glass funnel, to prevent loss of urine, for seventy minutes and the bladder was then emptied by means of massage. One-half of the urine so obtained was diluted to 500 c.c., rendered alkaline and its phenolsulphonephthalein content was determined colorimetrically, using 1 c.c. of the original phenolsulphonephthalein solution, diluted to one liter, as a standard. The determination was made exactly as described by Rowntree and Geraghty,[7] using their modification of the Autenrieth-Königsberger colorimeter. The other half of the urine was used in connection with the examination for casts and tests for albumin. Nitric acid was used throughout for the albumin tests.

The kidney tissues were preserved for the histological examination (a) in Zenker's solution, (b) in formalin (10 per cent.), and were then stained (a) with eosin and methylene blue and (b) with scharlach R.

The blood of normal rabbits contains about 30 mg. of total non-protein nitrogen and about 13 mg. of urea nitrogen per 100 gm.[7] The phenolsulphonephthalein excretion in such rabbits amounts to 60 per cent. or over in seventy minutes when 6 milligrams are injected under the described conditions.

7. Rowntree and Geraghty: THE ARCHIVES INT. MED., 1912, ix, 284.

EXPERIMENTS

Experiment 1.—Rabbit 518.[8] Urine normal. Died[9] on the *first day* after the administration of the uranium nitrate. (Acute pulmonary edema.)

First day (wt. 1,520 gm.): Phenolsulphonephthalein excretion.....70 per cent.
Administered 3 mg. uranium nitrate.
Second day (wt. 1,535 gm.): Phenolsulphonephthalein excretion....30 per cent.
Urine: albumin trace; epithelial cells but no casts.
Blood: non-protein nitrogen (per 100 gm.).....41 mg.
Blood: urea nitrogen (per 100 gm.).....13 mg.
Histological Findings: Glomeruli slightly congested. Tubules negative; no excess of fat.

Experiment 2.—Rabbit 520. Urine normal. Killed on the *second day* after the administration of uranium nitrate.

First day (wt. 1,140 gm.): Phenolsulphonephthalein excretion.....54 per cent.
Administered 3 mg. uranium nitrate.
Second day (wt. 1,140 gm.): Phenolsulphonephthalein excretion....46 per cent.
Urine: albumin trace; epithelial cells present; no casts.
Third day (wt. 1,140 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin trace; epithelial cells and a few casts.
Blood: non-protein nitrogen (per 100 gm.).....32 mg.
Blood: urea nitrogen (per 100 gm.).....13 mg.
Histological Findings: Glomeruli slightly congested. Necrosis with desquamation of epithelium in some tubules. No excess of fat. A few areas of round cell infiltration.

Experiment 3.—Rabbit 521. Faint trace of albumin in urine, no casts. Killed on the *third day* after the administration of the uranium nitrate.

First day (wt. 1,350 gm.): Phenolsulphonephthalein excretion.....68 per cent.
Administered 3 mg. uranium nitrate.
Second day (wt. 1,300 gm.): Phenolsulphonephthalein excretion....31 per cent.
Urine: albumin faint trace; epithelial cells; no casts.
Third day (wt. 1,210 gm.): Phenolsulphonephthalein excretion....20 per cent.
Urine: albumin trace; epithelial cells; no casts.
Fourth day (wt. 1,160 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin trace; epithelial cells; casts.
Blood: non-protein nitrogen (per 100 gm.).....116 mg.
Blood: urea nitrogen (per 100 gm.)..... 69 mg.
Histological Findings: Glomeruli negative; considerable necrosis and desquamation of certain tubules; necrotic material in lumen of other tubules.

Experiment 4.—Rabbit 523. Urine normal. Killed on the *fourth day* after the administration of the uranium nitrate.

First day (wt. 1,400 gm.): Phenolsulphonephthalein excretion.....75 per cent.
Administered 3 mg. uranium nitrate.
Second day (wt. 1,390 gm.): Phenolsulphonephthalein excretion....58 per cent.
Urine: albumin trace; epithelial cells present; no casts.
Third day (wt. 1,330 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin trace; epithelial cells present; no casts.
Fourth day (wt. 1,370 gm.): Phenolsulphonephthalein excretion.....trace
Urine: practically anuric.
Fifth day (wt. 1,370 gm.): Phenolsulphonephthalein excretion.....trace
Urine: no albumin; many casts.
Blood: non-protein nitrogen (per 100 gm.).....142 mg.
Blood: urea nitrogen (per 100 gm.).....109 mg.

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8. In the following experiments the animal numbers are those recorded in the laboratory of Theory and Practice.

9. Blood had not clotted and was collected from the right pleural cavity.

Histological Findings: Glomeruli negative. Necrotic epithelium in many tubules; disappearance of epithelium in certain tubules; necrotic material and casts in others. Slight excess of fat in necrotic material. A few areas of round cell infiltration.

Experiment 5.—Rabbit 524. Urine normal. Found dead on the morning of the *fifth day* after the administration of uranium. Blood[10] taken from the dead animal for analysis.

First day (wt. 1,440 gm.): Phenolsulphonephthalein excretion....68 per cent.
Administered 3 mg. uranium nitrate.
Second day (wt. 1,425 gm.): Phenolsulphonephthalein excretion...45 per cent.
Urine: albumin trace; epithelial cells present; no casts.
Third day (wt. 1,400 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin large trace; epithelial cells present; few casts.
Fourth day (wt. 1,400 gm.): Phenolsulphonephthalein excretion.....trace
Urine: practically anuric.
Fifth day (wt. 1,380 gm.): Phenolsulphonephthalein excretion.....anuric
Sixth day: Blood: non-protein nitrogen (per 100 gm.).....256 mg.
Blood: urea nitrogen (per 100 gm.).....172 mg.

Histological Findings: Edema and congestion of glomeruli. Necrotic epithelium in many tubules; disappearance of epithelium in many; necrotic material and casts in others. Slight excess of fat in necrotic material and tubular epithelium. No round cell infiltration.

Experiment 6.—Rabbit 532. Urine normal. Killed on the *fifth day* after the administration of the uranium.

First day (wt. 1,260 gm.): Phenolsulphonephthalein excretion.....68 per cent.
Administered 3 mg. uranium nitrate.
Fourth day (wt. 1,175 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin large trace; many casts.
Sixth day (wt. 1,225 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin trace; a few casts.
Blood: non-protein nitrogen (per 100 gm.).....216 mg.
Blood: urea nitrogen (per 100 gm.).....125 (?) mg.

Histological Findings: Glomeruli negative. Disappearance of epithelium in certain tubules; regeneration of epithelium in others; necrotic material and casts in some. A few areas of round cell infiltration.

Experiment 7.—Rabbit 528. Urine normal. Killed on the *seventh day* after the administration of the uranium.

First day (wt. 1,750 gm.): Phenolsulphonephthalein excretion.....79 per cent.
Administered 3 mg. uranium nitrate.
Second day (wt. 1,720 gm.): Phenolsulphonephthalein excretion....49 per cent.
Urine: albumin trace; epithelial cells present; no casts.
Third day (wt. 1,660 gm.): Phenolsulphonephthalein excretion....19 per cent.
Urine: albumin large trace; epithelial cells present; a few casts.
Fourth day (wt. 1,660 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin trace; few casts.
Fifth day (wt. 1,620 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin trace; many casts.
Sixth day (wt. 1,500 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin trace; many casts.
Seventh day (wt. 1,470 gm.): Phenolsulphonephthalein excretion...10 per cent.
Urine: albumin slight trace; many casts.
Eighth day (wt. 1,460 gm.): Phenolsulphonephthalein excretion...38 per cent.
Urine: albumin negative; many casts.
Blood: non-protein nitrogen (per 100 gm.).....92 mg.
Blood: urea nitrogen (per 100 gm.).....62 mg.

10. Blood had not clotted and was taken from right pleural cavity.

Histological Findings: Glomeruli negative. Necrotic epithelium in a few tubules; considerable regeneration in some tubules. Necrotic material and casts in collecting tubules. Fat in necrotic material and in the epithelium of some tubules. Round cell infiltration in many areas.

Experiment 8.—Rabbit 529. Urine normal. Killed on the *eighth day* after the administration of uranium nitrate.

First day (wt. 1,380 gm): Phenolsulphonephthalein excretion.....72 per cent.
Administered 3 mg. uranium nitrate.
Second day: Phenolsulphonephthalein excretion.....59 per cent.
Urine: albumin slight trace; no casts; epithelium present.
Third day (wt. 1,310 gm.): Phenolsulphonephthalein excretion.....2 per cent.
Urine: albumin trace; epithelial cells present; a few casts.
Fourth day (wt. 1,310 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin large trace; epithelial cells; rare casts.
Fifth day (wt. 1,340 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin large trace; many casts.
Sixth day (wt. 1,360 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin none; few casts.
Seventh day (wt. 1,260 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin slight trace; many casts.
Eighth day (wt. 1,230 gm.): Phenolsulphonephthalein excretion....14 per cent.
Urine: albumin slight trace; many casts.
Ninth day (wt. 970 gm.): Phenolsulphonephthalein excretion.....12 per cent.
Urine: albumin negative; many casts.
Blood: non-protein nitrogen (per 100 gm.).....266 mg.
Blood: urea nitrogen (per 100 gm.).....200 mg.

Histological Findings: A few glomeruli show hyaline droplets. Considerable necrotic epithelium in tubules; regeneration in some. Necrotic material and casts in tubules. No excess of fat. No round cell infiltration.

Experiment 9.—Rabbit 533. Urine normal. Killed on the *tenth day* after the administration of uranium nitrate.

First day (wt. 1,180 gm.): Phenolsulphonephthalein excretion.....66 per cent.
Administered 2 mg. uranium nitrate.
Fourth day (wt. 1,125 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin trace; epithelial cells and some casts present.
Sixth day (wt. 1,080 gm.): Phenolsulphonephthalein excretion.....1 per cent.
Urine: albumin trace; many casts.
Eighth day (wt. 1,010 gm.): Phenolsulphonephthalein excretion....42 per cent.
Urine: albumin slight trace; many casts.
Ninth day (wt. 975 gm.): Phenolsulphonephthalein excretion.....34 per cent.
Urine: no albumin; a few casts.
Eleventh day (wt 910 gm.): Phenolsulphonephthalein excretion....60 per cent.
Urine: normal.
Blood: non-protein nitrogen (per 100 gm.).....59 mg.
Blood: urea nitrogen (per 100 gm.).....43 mg.

Histological Findings: Glomeruli negative. Tubules negative except for a few showing regeneration. No excess of fat. Numerous areas of round cell infiltration.

From Experiments 1 to 9, recorded above, it is evident that the excretion of phenolsulphonephthalein is very much diminished on the day following the administration of uranium nitrate, and that by the second or third day the excretion is reduced to a mere trace or ceases altogether. The accumulation of the nitrogenous waste products in the blood is of course gradual, and would naturally be very slow in a herbiv-

orous animal living chiefly on carrots. After the third day, however, the accumulation of non-protein nitrogen and urea in the blood reaches extraordinary figures. The fact that the animals often abstained from eating during this period probably resulted in their using up of their supply of glycogen, and this would greatly accelerate the protein destruction and the accumulation of the nitrogenous products. Variations in the accumulation during the anuric period are doubtless chiefly due to variations in the consumption of carbohydrates. As the retention of nitrogen increases the proportion represented by the urea becomes increasingly larger. The histological evidence of injury to the kidney from the uranium did not appear until the second day after the administration of the uranium. Traces of albumin in the urine appeared, on the other hand, uniformly at the end of the first day, though casts did not occur until the third day. After the third day, when extensive necrosis of the tubular epithelium is found, the enormous accumulation of nitrogen in the blood and the more or less complete retention of the phenolsulphonephthalein also revealed a condition of severe nephritis and renal insufficiency.

The transitory character of uranium nephritis is illustrated by the last three experiments. After the sixth or seventh day the phenolsulphonephthalein excretion begins to improve. The improvement, however, may not be quite so rapid as is indicated by the percentage of the phenolsulphonephthalein excretion recorded in the tables, because relatively large amounts of the chemical may have accumulated in the animals as a result of the repeated injections during several preceding days when practically none of it was eliminated. The retention nitrogen in the blood, therefore, remains very high, even after there has been an apparently marked improvement in the phenolsulphonephthalein excretion. By the eleventh day the kidney appears practically normal again, but the non-protein nitrogen and urea are still slightly increased. The urine is free from albumin and casts, and the phenolsulphonephthalein excretion has returned practically to normal. It is evident from this series of animals that these tests of blood and urine varied with the intensity of the nephritis and in general paralleled each other.

In order to compare more accurately the relation of the accumulation of the non-protein nitrogenous products in the blood with the excretion of phenolsulphonephthalein at different stages of acute nephritis, a second series of experiments was made. In this series the blood was collected every two or three days from the ear veins and on the same days the phenolsulphonephthalein test was applied. Experiments 10 to 19, recorded below, give the salient points of these observations.

Experiment 10.—Rabbit 669. Urine normal; blood normal. Animal found dead on the fifth day after the administration of the uranium nitrate.

First day (wt. 1,850 gm.): Phenolsulphonephthalein excretion.....75 per cent.
Blood: non-protein nitrogen (per 100 gm.).....29 mg.
Administered 3 mg. uranium nitrate.

Third day (wt. 1,830 gm.): Phenolsulphonephthalein excretion.....trace
Urine: albumin trace; epithelial cells; no casts.
Blood: non-protein nitrogen (per 100 gm.).....49 mg.
Blood: urea nitrogen (per 100 gm.).....30 mg.

Fifth day: Phenolsulphonephthalein excretion.....anuric
Blood: non-protein nitrogen (per 100 gm.).....120 mg.
Blood: urea nitrogen (per 100 gm.)..... 82 mg.

Experiment 11.—Rabbit 671. Urine normal. High initial nitrogen in the blood. Found dead on the seventh day after the administration of the uranium.

First day (wt. 1,580 gm.): Phenolsulphonephthalein excretion.....80 per cent.
Blood: non-protein nitrogen (per 100 gm.).....45 mg.
Blood: urea nitrogen (per 100 gm.).....26 mg.
Administered 1.25 mg. uranium nitrate.

Fourth day (wt. 1,520 gm.): Phenolsulphonephthalein excretion.....trace
Blood: non-protein nitrogen (per 100 gm.).....80 mg.
Blood: urea nitrogen (per 100 gm.).....55 mg.
Urine: albumin large trace; a few casts; epithelial cells.

Seventh day (wt. 1,400 gm.): Phenolsulphonephthalein excretion.....trace
Blood: non-protein nitrogen (per 100 gm.).....250 mg.
Blood: urea-nitrogen (per 100 gm.).....201 mg.
Urine: albumin trace; sediment not done.

Experiment 12.—Rabbit 670. Urine normal; high initial nitrogen in blood. Found dead on the ninth day after the administration of the uranium.

First day (wt. 1,270 gm.): Phenolsulphonephthalein excretion.....71 per cent.
Blood: non-protein nitrogen (per 100 gm.).....45 mg.
Blood: urea nitrogen (per 100 gm.).....25 mg.
Administered 1.25 mg. uranium nitrate.

Fourth day (wt. 1,250 gm.): Phenolsulphonephthalein excretion.....trace
Blood: non-protein nitrogen (per 100 gm.).....100 mg.
Blood: urea nitrogen (per 100 gm.)..... 74 mg.
Urine: albumin trace; a few casts; epithelium.

Seventh day (wt. 1,060 gm.): Phenolsulphonephthalein excretion....12 per cent.
Blood: non-protein nitrogen (per 100 gm.).....190 mg.
Blood: urea nitrogen (per 100 gm).....161 mg.
Urine: many casts; no albumin.

Ninth day (wt. 950 gm.): Phenolsulphonephthalein excretion.....43 per cent.
Blood: non-protein nitrogen (per 100 gm.).....95 mg.
Blood: urea nitrogen (per 100 gm.).....60 mg.
Urine: No albumin; many casts.

Experiment 13.—Rabbit 535. Urine normal, high initial nitrogen in the blood. Killed on the thirteenth day after the administration of uranium.

First day (wt. 1,620 gm): Phenolsulphonephthalein excretion....70 per cent.
Blood: non-protein nitrogen (per 100 gm.).....50 mg.
Blood: urea nitrogen (per 100 gm.).....30 mg.
Administered 1.25 mg. uranium nitrate.

Fourth day (wt. 1,630 gm.): Phenolsulphonephthalein excretion....51 per cent
Blood: non-protein nitrogen (per 100 gm.).....50 mg.
Blood: urea nitrogen (per 100 gm.).....23 mg.
Urine: albumin trace; epithelial cells and casts absent.

Seventh day (wt. 1,630 gm.): Phenolsulphonephthalein excretion...48 per cent.
Blood: non-protein nitrogen (per 100 gm.).....50 mg.
Blood: urea nitrogen (per 100 gm.)..... 25 mg.
Urine: albumin large trace; sediment negative.

Ninth day (wt. 1,600 gm.): Phenolsulphonephthalein excretion...62 per cent.
Blood: non-protein nitrogen (per 100 gm.).....30 mg.
Blood: urea nitrogen (per 100 gm.).....16 mg.
Urine: albumin negative; a few casts.

Eleventh day (wt. 1,660 gm.): Phenolsulphonephthalein excretion..70 per cent.
Blood: non-protein nitrogen (per 100 gm.).....30 mg.
Blood: urea nitrogen (per 100 gm.).....16 mg.
Urine: no albumin; rare cast.

Fourteenth day (wt. 1,660 gm.): Phenolsulphonephthalein excretion, 69 per cent.
Blood: non-protein nitrogen (per 100 gm.).....32 mg.
Blood: urea nitrogen (per 100 gm.).....20 mg.
Urine: normal.

Histological Findings: Glomeruli negative. Tubules negative; no excess of fat; no cellular infiltration.

Experiment 14.—Rabbit 534. Pregnant. Urine and blood normal. Killed on the thirteenth day after the administration of uranium.

First day (wt. 2,220 gm.): Phenolsulphonephthalein excretion.....70 per cent.
Blood: non-protein nitrogen (per 100 gm.).....30 mg.
Administered 3 mg. uranium nitrate.

Third day (wt. 2,210 gm.): Phenolsulphonephthalein excretion.....trace
Blood: non-protein nitrogen (per 100 gm.).....45 mg.
Blood: urea nitrogen (per 100 gm.).....30 mg.
Urine: albumin large trace; epithelial cells; no casts.

Fifth day: Phenolsulphonephthalein excretion.....trace
Blood: non-protein nitrogen (per 100 gm.).....112 mg.
Blood: urea nitrogen (per 100 gm.)..... 82 mg.
Urine: albumin trace; epithelium; many casts.

Seventh day (wt. 2,100 gm.): Phenolsulphonephthalein excretion....25 per cent.
Blood: non-protein nitrogen (per 100 gm.).....102 mg.
Blood: urea nitrogen (per 100 gm.)..... 77 mg.
Urine: albumin trace; many casts.

Ninth day (wt. 1,190 gm.): Phenolsulphonephthalein excretion.....40 per cent.
Blood: non-protein nitrogen (per 100 gm.).....59 mg.
Blood: urea nitrogen (per 100 gm.).....40 mg.
Urine not examined.

Tenth day: Phenolsulphonephthalein excretion.....46 per cent.
Blood: non-protein nitrogen (per 100 gm.).....45 mg.
Blood: urea nitrogen (per 100 gm.).....25 mg.
Urine: albumin trace; many casts.

Twelfth day (wt. 1,800 gm.): Phenolsulphonephthalein excretion...31 per cent.
Blood: non-protein nitrogen (per 100 gm.).....35 mg.
Blood: urea nitrogen (per 100 gm.).....23 mg.
Urine not examined.

Fourteenth day: Phenolsulphonephthalein excretion.....36 per cent.
Blood: non-protein nitrogen (per 100 gm.).....57 mg.
Blood: urea nitrogen (per 100 gm.).....30 mg.
Urine: albumin trace; no casts.

Histological Findings: Glomeruli negative. A few tubules showed regeneration and a few showed casts. Slight amount of fat in some tubules. No cellular infiltration.

Experiment 15.—Rabbit 536. Urine normal; blood normal. Killed on the eighteenth day after the administration of uranium.

First day (wt. 1,620 gm.): Phenolsulphonephthalein excretion.....78 per cent.
Blood: non-protein nitrogen (per 100 gm.).....31 mg.
Blood: urea nitrogen (per 100 gm.).....15 mg.
Administered 1.25 mg. uranium nitrate.

Fourth day (wt. 1,570 gm.): Phenolsulphonephthalein excretion.....trace
Blood: non-protein nitrogen (per 100 gm.).....50 mg.
Blood: urea nitrogen (per 100 gm.).....23 mg.
Urine: albumin trace; epithelium; a few casts.

Seventh day (wt. 1,530 gm.): Phenolsulphonephthalein excretion....8 per cent.
Blood: non-protein nitrogen (per 100 gm.).....97 mg.
Blood: urea nitrogen (per 100 gm.).....74 mg.
Urine: albumin trace; many casts.

Ninth day (wt. 1,460 gm.): Phenolsulphonephthalein excretion....52 per cent.
Blood: non-protein nitrogen (per 100 gm.).....47 mg.
Blood: urea nitrogen (per 100 gm.).....27 mg.
Urine: no albumin; many casts.

Eleventh day (wt. 1,630 gm.): Phenolsulphonephthalein excretion..58 per cent.
Blood: non-protein nitrogen (per 100 gm.).....40 mg.
Blood: urea nitrogen (per 100 gm.).....25 mg.
Urine: albumin trace; a few casts.

Fourteenth day (wt. 1,540 gm.): Phenolsulphonephthalein excretion, 65 per cent.
Blood: non-protein nitrogen (per 100 gm.).....32 mg.
Blood: urea nitrogen (per 100 gm.).....18 mg.
Urine: albumin negative; a few casts.

Sixteenth day: Phenolsulphonephthalein excretion.....62 per cent.
Blood: non-protein nitrogen (per 100 gm.).....32 mg.
Blood: urea nitrogen (per 100 gm.).....16 mg.
Urine: albumin negative; a few casts.

Nineteenth day (wt. 1,580 gm.): Phenolsulphonephthalein excretion, 72 per cent.
Blood: non-protein nitrogen (per 100 gm.).....26 mg.
Blood: urea nitrogen (per 100 gm.).....13 mg.
Urine: normal.

Histological Findings: Glomeruli negative. Tubules negative; no excess of fat; no cellular infiltration.

Experiment 16.—Rabbit 681. Urine: albumin a trace; no casts. Blood normal.

First day (wt. 2,130 gm.): Phenolsulphonephthalein excretion.....62 per cent.
Blood: non-protein nitrogen (per 100 gm.).....32 mg.
Blood: urea nitrogen (per 100 gm.).....21 mg.
Administered 3 mg. uranium nitrate.

Third day (wt. 2,080 gm.): Phenolsulphonephthalein excretion.....14 per cent.
Blood: non-protein nitrogen (per 100 gm.).....47 mg.
Blood: urea nitrogen (per 100 gm.).....25 mg.
Urine: albumin trace; epithelial cells; no casts.

Fifth day (wt. 1,940 gm.): Phenolsulphonephthalein excretion.....trace
Blood: non-protein nitrogen (per 100 gm.).....85 mg.
Blood: urea nitrogen (per 100 gm.).....60 mg.
Urine: albumin trace; epithelial cells; no casts.

Seventh day (wt. 1,930 gm.): Phenolsulphonephthalein excretion...23 per cent.
Blood: non-protein nitrogen (per 100 gm.).....90 mg.
Blood: urea nitrogen (per 100 gm.).....68 mg.
Urine: albumin trace; rare cast.

Eighth day (wt. 1,900 gm.): Phenolsulphonephthalein excretion....40 per cent.
Blood: non-protein nitrogen (per 100 gm.).....80 mg.
Blood: urea nitrogen (per 100 gm.).....56 mg.
Urine: albumin trace; casts.

Tenth day (wt. 1,770 gm.): Phenolsulphonephthalein excretion.....58 per cent.
Blood: non-protein nitrogen (per 100 gm.).....54 mg.
Blood: urea nitrogen (per 100 gm.).....40 mg.
Urine: albumin slight trace; many casts.

Twelfth day (wt. 1,800 gm.): Phenolsulphonephthalein excretion....64 per cent.
Blood: non-protein nitrogen (per 100 gm.).....36 mg.
Blood: urea nitrogen (per 100 gm.).....19 mg.
Urine: no albumin; many casts.

Fourteenth day (wt. 1,750 gm.): Phenolsulphonephthalein excretion, 48 pct. (?)
Blood: non-protein nitrogen (per 100 gm.).....27 mg.
Blood: urea nitrogen (per 100 gm.).....18 mg.
Urine: no albumin; few casts.

Sixteenth day: Phenolsulphonephthalein excretion.....68 per cent.
Blood: non-protein nitrogen (per 100 gm.).....28 mg.
Blood: urea nitrogen (per 100 gm.).....19 mg.
Urine: no albumin; few casts.

Experiment 17.—Rabbit 679. Urine normal; blood normal.

First day (wt. 2,620 gm.): Phenolsulphonephthalein excretion.....71 per cent.
Blood: non-protein nitrogen (per 100 gm.).....28 mg.
Blood: urea nitrogen (per 100 gm.).....18 mg.
Administered 3 mg. uranium nitrate.

Third day (wt. 2,630 gm.): Phenolsulphonephthalein excretion.....52 per cent.
Blood: non-protein nitrogen (per 100 gm.).....28 mg.
Blood: urea nitrogen (per 100 gm.).....18 mg.
Urine: albumin trace; epithelium; no casts.

Fifth day (wt. 2,470 gm.): Phenolsulphonephthalein excretion.....14 per cent.
Blood: non-protein nitrogen (per 100 gm.).....52 mg.
Blood: urea nitrogen (per 100 gm.).....31 mg.
Urine: albumin trace; a few casts.

Seventh day (wt. 2,570 gm.): Phenolsulphonephthalein excretion...18 per cent.
Blood: non-protein nitrogen (per 100 gm.).....55 mg.
Blood: urea nitrogen (per 100 gm.).....32 mg.
Urine: albumin large trace; a few casts.

Eighth day (wt. 2,500 gm.): Phenolsulphonephthalein excretion....37 per cent.
Blood: non-protein nitrogen (per 100 gm.).....55 mg.
Blood: urea nitrogen (per 100 gm.).....32 mg.
Urine: albumin trace; casts.

Tenth day (wt. 2,600 gm.): Phenolsulphonephthalein excretion....69 per cent.
Blood: non-protein nitrogen (per 100 gm.).....50 mg.
Blood: urea nitrogen (per 100 gm.).....37 mg.
Urine: no albumin; few casts.

Twelfth day (wt. 2,500 gm.): Phenolsulphonephthalein excretion....56 pct. (?)
Blood: non-protein nitrogen (per 100 gm.).....35 mg.
Blood: urea nitrogen (per 100 gm.).....19 mg.
Urine: albumin negative; a few casts.

Fourteenth day (wt. 2,575 gm.): Phenolsulphonephthalein excretion, 75 per cent.
Blood: non-protein nitrogen (per 100 gm.).....27 mg.
Blood: urea nitrogen (per 100 gm.).....18 mg.
Urine normal.

Experiment 18.—Rabbit 680. Urine normal; blood normal.

First day (wt. 2,180 gm.): Phenolsulphonephthalein excretion.....68 per cent.
Blood: non-protein nitrogen (per 100 gm.).....29 mg.
Blood: urea nitrogen (per 100 gm.).....18 mg.
Administered 3 mg. uranium nitrate.
Third day (wt. 2,150 gm.): Phenolsulphonephthalein excretion.....60 per cent.
Blood: non-protein nitrogen (per 100 gm.).....35 mg.
Blood: urea nitrogen (per 100 gm.).....20 mg.
Urine: albumin trace; no epithelium; no casts.
Fifth day (wt. 2,030 gm.): Phenolsulphonephthalein excretion.....49 per cent.
Blood: non-protein nitrogen (per 100 gm.).....35 mg.
Blood: urea nitrogen (per 100 gm.).....19 mg.
Urine: albumin trace; epithelial cells present.
Seventh day (wt. 2,070 gm.): Phenolsulphonephthalein excretion...62 per cent.
Blood: non-protein nitrogen (per 100 gm.).....35 mg.
Blood: urea nitrogen (per 100 gm.).....20 mg.
Urine: albumin trace; casts.
Eighth day (wt. 2,000 gm.): Phenolsulphonephthalein excretion....64 per cent.
Blood: non-protein nitrogen (per 100 gm.).....32 mg.
Blood: urea nitrogen (per 100 gm.).....19 mg.
Urine: albumin negative; casts.
Tenth day (wt. 2,020 gm.): Phenolsulphonephthalein excretion.....67 per cent.
Blood: non-protein nitrogen (per 100 gm.).....26 mg.
Blood: urea nitrogen (per 100 gm.).....13 mg.
Urine: no albumin; few casts.

Experiment 19.—Rabbit 682. Urine normal; blood normal.

First day (wt. 2,050 gm.): Phenolsulphonephthalein excretion....60 per cent.
Blood: non-protein nitrogen (per 100 gm.).....20 mg.
Blood: urea nitrogen (per 100 gm.).....18 mg.
Administered 3 mg. uranium nitrate.
Third day (wt. 2,030 gm.): Phenolsulphonephthalein excretion.....25 per cent.
Blood: non-protein nitrogen (per 100 gm.).....29 mg.
Blood: urea nitrogen (per 100 gm.).....18 mg.
Urine: albumin trace; epithelium; no casts.
Fifth day (wt. 1,930 gm.): Phenolsulphonephthalein excretion.....trace
Blood: non-protein nitrogen (per 100 gm.).....55 mg.
Blood: urea nitrogen (per 100 gm.).....36 mg.
Urine: albumin trace; few casts.
Seventh day (wt. 1,935 gm.): Phenolsulphonephthalein excretion...18 per cent.
Blood: non-protein nitrogen (per 100 gm.).....62 mg.
Blood: urea nitrogen (per 100 gm.).....40 mg.
Urine: albumin trace; few casts.
Eighth day (wt. 1,870 gm.): Phenolsulphonephthalein excretion....38 per cent.
Blood: non-protein nitrogen (per 100 gm.).....72 mg.
Blood: urea nitrogen (per 100 gm.).....52 mg.
Urine: albumin trace; casts.
Tenth day (wt. 2,020 gm.): Phenolsulphonephthalein excretion.....55 per cent.
Blood: non-protein nitrogen (per 100 gm.).....47 mg.
Blood: urea nitrogen (per 100 gm.).....25 mg.
Urine: albumin negative; casts.
Twelfth day (wt. 1,970 gm.): Phenolsulphonephthalein excretion..62 per cent.
Blood: non-protein nitrogen (per 100 gm.).....37 mg.
Blood: urea nitrogen (per 100 gm.).....20 mg.
Urine: albumin trace; few casts.
Fourteenth day (wt. 1,950 gm.): Phenolsulphonephthalein excretion, 60 per cent.
Blood: non-protein nitrogen (per 100 gm.).....30 mg.
Blood: urea nitrogen (per 100 gm.).....18 mg.
Urine: albumin negative; few casts.

Three of the ten rabbits used in this series (Experiments 11, 12 and 13) showed a retention of nitrogen of 45, 45 and 50 milligrams, respectively, per 100 grams of blood, as against the normal figure of about 30 milligrams. This corresponds with what Folin and Denis have found in man, where the strictly normal is under 30 milligrams, yet where numerous exceptions are found among hospital patients. Two of these three rabbits died during the experiment, though they had been given only 1.25 milligrams uranium nitrate.

The correspondence between the results of the phenolsulphonephthalein tests and those of the blood analyses in Experiments 10 to 19 is quite remarkable, and in this series the significance of the former cannot have been obscured by accumulations of the drug in the body.

The correspondence is shown graphically in the accompanying charts, (a) with reference to a moderate degree of nephritis (Exp. 17), (b) with reference to severe nephritis (Exp. 16).

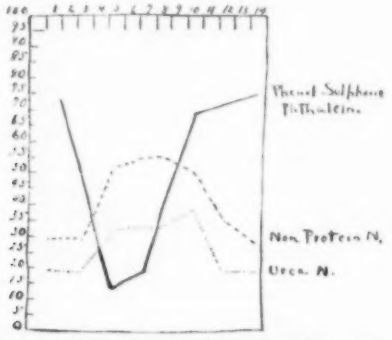

Chart 1.—Curves showing phenolsulphonephthalein excretion, non-protein nitrogen and urea nitrogen in a moderate nephritis, Experiment 17.

From the tables and the charts it is clear that at the beginning of the nephritis the phenolsulphonephthalein elimination drops more rapidly than the accumulation of non-protein nitrogen and the urea of the blood. During the course of the disease the height of the nitrogenous accumulation is reached from two to three days later than the lowest level of the phenolsulphonephthalein excretion. And after the kidney function has begun to improve, as shown by an increasing elimination of phenolsulphonephthalein in the urine, the retention nitrogen does not at once begin to recede, but may even continue to rise for a day or two, and does not reach the normal till a day or two after the phenolsulphonephthalein excretion. This is only what one must expect in view of the fact that the retention nitrogen represents the difference between that eliminated and that produced in the tissues, whereas the phenolsulphonephthalein

is an indication of the elimination alone. This is an essential difference between the two tests. The percentage of phenolsulphonephthalein excreted affords an index to the renal function at the time the test is made and the result is not visibly influenced by the length of time the kidney may have been in the condition indicated by the test. In the earliest stages of acute nephritis, such as is involved in these experiments, the nitrogen of the blood, on the other hand, does not necessarily reveal the intensity of the disease.

CONCLUSIONS

1. In acute uranium nephritis in rabbits the excretion of phenolsulphonephthalein in the urine and the amount of non-protein nitrogen and urea in the blood vary from the normal during the course of the nephritis and return to normal as the nephritis heals.

2. The degree of variation from the normal agrees on the whole with the amount of destruction demonstrated histologically in the kidney.

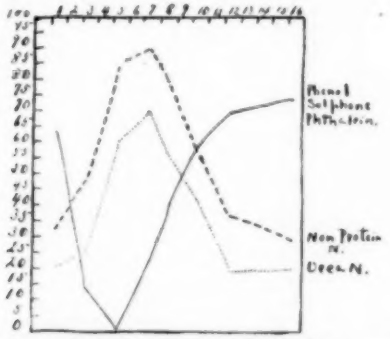

Chart 2.—Curves showing phenolsulphonephthalin excretion, non-protein nitrogen and urea nitrogen in severe nephritis, Experiment 16.

3. The phenolsulphonephthalein excretion in the urine drops rapidly to its lowest point and returns rapidly to normal with recovery of the kidney.

4. Non-protein nitrogen and urea accumulate gradually in the blood and return to normal gradually as the kidney recovers.

5. In general these tests parallel each other as indicators of renal function, but have this essential difference: the amount of phenolsulphonephthalein excretion shows the renal function at the moment; the amount of non-protein nitrogen and urea in the blood is rather a measure of an accumulating difference between the amounts of waste nitrogen produced in the metabolism and the amounts eliminated by the kidneys. The time element, the duration of the condition, is therefore an important factor in this test.

A CASE OF VEGETATIVE ENDOCARDITIS CAUSED BY A HITHERTO UNDESCRIBED SPIRILLUM. (SPIRILLUM SURATI, N. S.)*

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During the summer of 1911, we had the opportunity of observing the course of an unusual case of vegetative endocarditis, of recovering a hitherto undescribed spiral organism from the blood in pure culture on two separate occasions, and of confirming the clinical diagnosis and recovering the same organism from the blood, cardiac vegetations and liver at autopsy.

The causal relationship of this organism to the disease in question seems definite and well established.

CASE REPORT

The patient, a man aged 37, was admitted to the Presbyterian Hospital, July 13, 1911, in the service of Dr. Bovaird, to whom we are greatly indebted for permission to use the clinical records.

History.—There was nothing in the family history bearing on the case. As a baby he had pleurisy and at the age of 4 suffered from an attack of diphtheria. In 1896 he was ill for three weeks with a fever of unknown cause. Ten years later he suffered from cardiac decompensation due to aortic regurgitation. So far as known he had never suffered from rheumatism in any of its forms, unless the attack of fever in 1896 was of this nature. He never had malaria and gave no history of cough or loss of weight. For seven and one-half years previous to his admission he had been a missionary in the tropics, Surat Province, Bombay, India. He always took every precaution to avoid the various diseases prevalent there. Habits good. Not alcoholic.

Onset of present trouble March 12, 1911. He had been working hard in the intense heat of the tropics when he began to have chilly sensations, moderate fever and anorexia. No malarial organisms were found in his blood and in three days he was feeling well again.

Six days later, while on the ship for America, he had another similar attack, lasting for a few days. He did not take quinin. During the remainder of the trip he continued well.

In the middle of May he had a definite chill with fever lasting several hours. The following day he felt well. Two weeks later he had a similar attack. After this he was feverish at times and had slight morning anorexia and afternoon languor.

Two weeks before admission he had a very profuse night sweat and one week later he went to bed with a temperature of 104 F. An irregular temperature persisted, being normal in the morning and about 102 F. in the afternoon. He

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* From the Pathological Laboratory, Presbyterian Hospital, Columbia University, New York.

had regular night sweats but no cough or loss of weight. His sputum showed no tubercle bacilli. Appetite continued fairly good. Had no muscle or abdominal pain, no puffiness about the eyes and no urinary symptoms. Ever since going to the tropics he had had about two loose movements a day, containing a trace of fresh blood from hemorrhoids.

Examination.—Tall, [illegible] and hollow chested. Not dyspneic nor cyanotic. Looked worn and tire[illegible] than acutely ill. Eyes normal except for a little muddiness of the [illegible]. Face thin and sallow. Buccal mucous membrane, pharynx and tonsils normal. Lungs clear.

The apex impulse of the heart was of heaving character in the fifth space, 11 cm. to the left of the mid-line. The left limit of dulness was 1 cm. further to the left and the right limit at the right sternal margin. At the apex there were three murmurs: a short presystolic rumble; a soft, systolic blow, transmitted to the left axilla and heard over the whole precordium; a faint diastolic blow which increased in intensity as it was traced inwards. The pulmonic second sound was accentuated and there was a loud systolic murmur in this region. Over the aortic area there was a soft systolic murmur, transmitted upwards to the clavicle. Over the upper part of the sternum and along its left border, one heard a diastolic blow, loudest at the third costal cartilage.

The pulses were equal and of distinct Corrigan quality. The radial arteries were slightly thickened.

The abdomen was soft and showed nothing abnormal. The liver was not palpable. The sharp, firm edge of the spleen descended about 1 cm. below the costal margin on deep inspiration. The extremities and reflexes were normal. No petechiae were found.

The diagnosis on admission was chronic malaria and chronic cardiac valvular disease.

July 20. On admission the patient's strength was good. A blood count showed hemoglobin, 88 per cent.; red blood-cells, 4,200,000; white cells, 10,000; polymorphonuclears, 88 per cent.; lymphocytes, 11 per cent.; eosinophils, 1 per cent. No malarial organisms found on repeated examinations at various times of the day. Quinin had no effect; von Pirquet reaction negative. In reviewing the case at this time it seemed probable that the patient was suffering from acute infectious endocarditis.

July 23. Blood culture taken July 19 is sterile. Complains of pain in the calf of his leg. This is thought to be due to an embolus in a small artery. Two days ago the blood-count was: white cells, 15,200; polymorphonuclears, 82 per cent.; lymphocytes, 16 per cent.; eosinophils, 1 per cent.; basophils, 1 per cent. No malarial organisms found. No ova or parasites found in stool.

July 27. Fever still resists heavy doses of quinin. Physical examination remains unchanged.

August 1. Condition about the same. The irregular fever, sweats and chilliness continue. No petechiae have been found. Has two to four loose movements a day in which no ova or parasites have been found.

August 12. Has begun to lose appetite and has a little nausea. The blood count of August 3 showed: white cells, 11,500; polymorphonuclears, 77 per cent.; lymphocytes, 2 per cent.; large mononuclears, 15 per cent.; transitionals, 6 per cent. The cardiac dulness is 2 cm. further to the left.

August 17. Has been up and about for two or three days. Now has a little edema about the ankles. The blood count three days ago was: white cells, 21,000; polymorphonuclears, 90 per cent.; large mononuclears, 6 per cent.; lymphocytes, 4 per cent.; hemoglobin, 70 per cent. Wassermann reaction negative.

August 24. Grew steadily weaker and died this afternoon. Pulse continued rapid and irregular, heart dilated. Persistent abdominal distention. Labored respiration and moderate pulmonary edema. No petechiae found until the last two days when a few were seen over the thorax and one in the left conjunctiva. Two days before death the blood-count was: white cells, 36,400; polymorphonu-

clears, 88 per cent.; transitionals, 2 per cent.; lymphocytes, 9 per cent.; large mononuclears, 1 per cent.

During the course of the disease the temperature continued irregular. In the morning it would be normal or subnormal. In the afternoon the rise varied from 99.5 to 103 F. During the last two days it was continuously subnormal.

The pulse varied between 70 and 100 until near the end when it became much more rapid and irregular.

On repeated examinations the urine showed occasional hyaline casts and on one occasion a faint trace of albumin.

The patient was given four intravenous injections of collargol without any effect.

Necropsy.—The autopsy was performed six hours *post-mortem*. The body is that of a large framed man, showing considerable wasting. There is a little puffy edema over the dorsum of each foot. Scattered over the lower thorax and abdomen there are about a dozen small petechial spots and one is found in the left conjunctiva. The subcutaneous fat is pale and edematous.

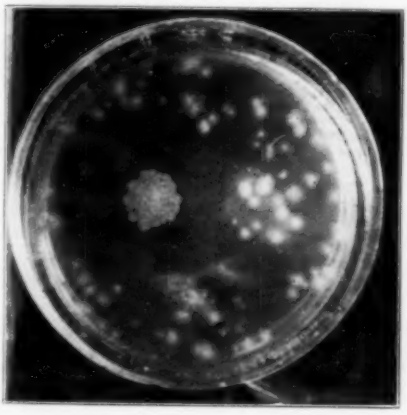

Fig. 1.—Cultures of the organism. (*Spirillum surati*). Appearance of glucose ascitic agar plate at the end of a week. Natural size.

On opening the thorax a broad precordial surface is exposed, the lung borders being well retracted to either side. Each pleural cavity is about one-third full of clear, light, amber-colored fluid. There are no adhesions present. The left lung weighs 500 gm. The surface is smooth. There are some areas of emphysema along the anterior border. The posterior and lower portions are congested. On section the lung is bright red in color and moderately edematous. It is air-containing and shows no areas of consolidation. The vessels are clear and the bronchi normal. The right lung weighs 560 gm. It is similar in all respects to the other except that it is a little more congested. The bronchial lymph-nodes are moderately pigmented but otherwise normal.

The pericardial sac contains about 300 c.c. of clear, light amber colored fluid. The pericardial surfaces are smooth and glistening. The right side of the heart is moderately enlarged and contains post-mortem clot. The heart weighs 550 gm. The tricupsid valve admits the tips of four fingers. The cusps are delicate and show no vegetations. The right ventricular wall measures 6 to 8 mm. in thickness. It is pale brown in color and somewhat muddy in appearance. Towards the lower part of the ventricle the muscle becomes more cloudy yellow in color.

The pulmonary valve is delicate and competent. The left side of the heart is moderately enlarged. The wall of the ventricle averages about 1.5 cm. in thickness. At a point about 6 cm. below the mitral ring the heart muscle becomes dull-yellow in color in contrast to the more reddish-brown appearance above. This area joins with the one in the right ventricle. The branches of the coronary artery supplying this region are perfectly patent and there is no evidence of any thrombus. The mitral valve appears as a button-hole slit. The cusps are fused together, thickened, moderately calcareous and somewhat retracted, forming a rigid ring of tissue and causing a marked stenosis of the orifice. Along the line of closure of the cusps and extending for a short distance on their upper and under surfaces, there are numerous small, soft vegetations with a little superficial ulceration. On opening the aorta, the site of the aortic valve is marked by a large, irregular, cauliflower-like vegetation of soft consistence. It is about the size of an English walnut and completely fills the orifice with the exception of small openings through the vegetation itself. One of these openings is in front, one behind and the third through the middle of the mass. The cusps are much distorted and fused together. The vegetations do not involve the wall of the heart, the papillary muscles or chordae tendinae. At the base of the aorta there are a few streaks of yellowish atheroma. The rest of the aorta is elastic

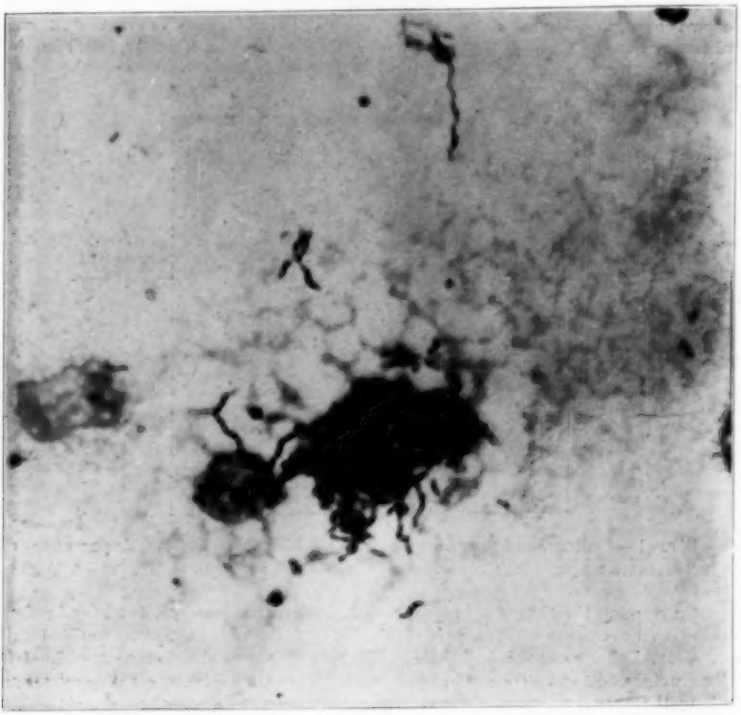

Fig. 2.—Stained preparation from the original blood-culture at the end of eight days. (Magnification 1-1900.)

and moderately smooth except in the lower portion where there are a few chalky deposits. The openings of the coronary arteries are free from sclerosis. In their course they show a little scattered atheroma, but at no point is this marked nor is their lumen obstructed in any way.

The abdomen contains about 500 c.c. of clear, amber fluid. The peritoneal surfaces are smooth and glistening. The spleen weighs 450 gm. It is not adherent to the surrounding structures. On section it is dark purplish-red in color and

Fig. 3.—Stained preparation from the original blood-culture at the end of two weeks. (Magnification 1-1900.)

the splenic pulp is very firm, the cut surface remaining perfectly flat. There are no infarcts present. The liver weighs 1,650 gm. Its surface is smooth, consistence firm and color muddy brown. On section it has the appearance of chronic passive congestion. The gall-bladder and ducts are normal. The left kidney

weighs 200 gm. Its capsule is slightly adherent. The surface is smooth and of normal color. The kidney is firm. Near the upper pole there is a small infarct. On section the kidney has the appearance of chronic passive congestion and cloudy swelling. The pelvis and ureter are normal. The right kidney weighs 170 gm. It is similar in all respects to the other except that there are no infarcts present. The adrenals appear perfectly normal. The pancreas is firm and of normal color. At its widest part it measures 6 cm. It appears normal on section. The ducts are normal. The esophagus, stomach and intestines show no change. The mesenteric lymph-nodes are slightly enlarged. The prostate and bladder are normal. The brain was not examined.

Fig. 4.—Preparation from a deep ascitic glucose agar culture forty-eight hours old. Dark field illumination. (Magnification 1-1100.)

Microscopical Examination.—Heart muscle: The pericardium is a little edematous and shows some infiltration with mononuclear wandering cells. The muscle fibers just beneath the pericardium and endocardium are pale, swollen and degenerated. In general, the heart muscle has the appearance of cloudy swelling. Corresponding with the dirty yellow area described on gross examination, the picture is quite different. Here one sees many polymorphonuclear leukocytes scattered diffusely between the muscle bundles and even between the individual fibers. They are seen especially about the vessels and these are congested and show many polymorphonuclear leukocytes in the lumina. Some of the very small vessels are thrombosed. Many of the muscle fibers are quite granular and show no nuclei.

Mitral and Aortic Vegetations: Sections show structureless, pinkish material with some fibrin, blood-platelets and polymorphonuclear leukocytes. In some areas there are small hemorrhages. Just beneath the free surfaces and extending irregularly throughout the vegetations, one sees many dense masses and scattered clumps of bacteria. With the hemotoxylin-eosin stain it is difficult to make out the form of these organisms.

Lungs: Show chronic passive congestion and edema.

Spleen: Shows nothing more than well marked chronic passive congestion.

Liver: The same process is very marked in the liver, fully one-half of each lobule being affected. Otherwise, the liver cells show cloudy swelling and some fatty infiltration.

Fig. 5.—From an ascitic fluid tissue culture covered with paraffin oil. Ten days old. Dark field illumination. (Magnification 1-1100.)

Kidney: There is a typical infarct. There is also general congestion and cloudy swelling. The glomeruli were carefully searched for any evidences of the lesions which have been described as occurring with subacute vegetative endocarditis, but no such changes were found.

Pancreas: Normal.

Adrenals: Normal.

SPECIMENS PREPARED WITH LEVADITI STAIN

Sections of the vegetations from the aortic and mitral valves show a very striking picture. Scattered through the sections are dense masses of bacteria stained black. In places these organisms are so densely packed together that it

is quite impossible to make out their forms. Where they are less numerous, one can see many slightly curved bacilli, comma-and S-shaped forms, thicker than in the preparations from cultures, owing to the impregnation with silver salt. Here and there in these clumps one can make out perfectly definite spirilla of varying lengths. In other portions of the sections, one finds small clumps and isolated organisms. Some of these appear as beautiful spirilla of from eight to ten turns; others as commas and S-shaped forms.

A study of these sections shows the organism appearing in its various forms as in cultures.

Sections of the liver, spleen, heart-muscle, kidney and pancreas stained by the same method fail to show any of the organisms.

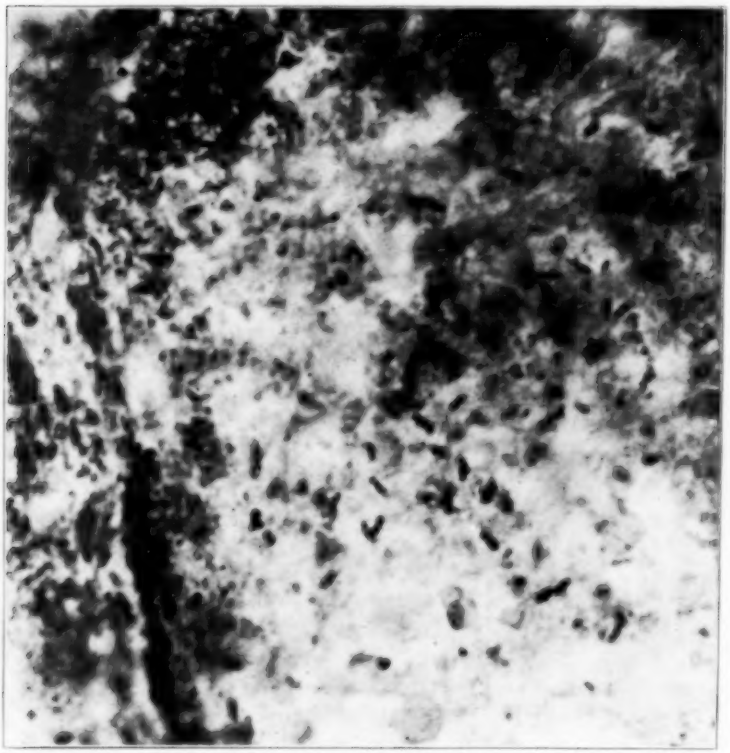

Fig. 6.—Section of aortic vegetation stained by the Levaditi method. It shows numerous small S and comma forms. (Magnification 1-1900.)

CULTURES TAKEN AT AUTOPSY

Cardiac Vegetations: Pure growth of the spirillum.

Heart Blood: Mixed growth of spirilla and colon bacilli.

Liver: Two cultures showed a pure growth of the spirillum and one culture gave only the colon bacillus.

Pericardial Fluid: Colon bacillus.

Pleural Fluid: Sterile.

Ascitic Fluid: Sterile.

Spleen: Sterile.

Smears: These were made from the aortic and mitral valve vegetations. They showed numerous perfectly definite spirilla of varying lengths and no other organisms.

BLOOD-CULTURES AND DESCRIPTION OF THE ORGANISM

A blood-culture taken July 19 remained sterile for five days, was so reported and discarded. It was the custom in the laboratory at that time to keep all blood-cultures for five days, making the final observations and reports at the end of that time.

Fig. 7.—Section of aortic vegetation stained by the Levaditi method showing a short spirillum and a few of the smaller forms thickly coated with the silver salt. (Magnification 1-1900.)

The second culture was taken August 13. Different quantities of the patient's blood were added to each of three flasks of nutrient broth (250 c.c.) faintly alkaline to litmus. For four days the cultures remained sterile and were not opened. The supernatant fluid was clear and the blood and clot were unchanged. On the fifth day the cultures were examined and found sterile. The flasks were

not discarded, however. On the sixth day, the supernatant fluid in all three flasks was distinctly turbid and there was slight hemolysis present. On examining hanging drop preparations, no free organisms could be seen, but here and there were small clumps of granular material. These clumps resembled the picture seen in cultures incubated for four or five days where the red cells have begun to break up, forming little masses of debris. On the following day, the

Fig. 8.—Section of aortic vegetation stained by the Levaditi method. It shows a spirillum just to the left of the middle, rather thickly coated with silver salt. In the upper right hand corner there is an S-shaped form and in the lower left hand corner a comma form. (Magnification 1-1900.)

seventh from the time of taking the cultures, hanging drop preparations showed free spirilla making their way actively across the field. There were also many of the granular clumps similar to those seen on the previous day. From the periphery of these clumps, numerous spirilla projected and occasionally one could

be seen to break away and move very actively across the field. It was at first thought that some very unusual contamination had taken place. The two other flasks were examined and a similar picture found in each. The smaller spirilla were very actively motile, traveling across the field so rapidly and at such varying depths that it was very difficult to follow them. The longer forms were rather sluggish and stiff and showed very little flexibility of their bodies.

Drops of the broth culture stained with methylene blue or carbol-fuchsin showed the clumps already referred to. (See illustration.) These consisted of tangled masses of organisms, most of which appeared in the form of small, slightly curved bacilli, commas and S-shaped forms. Some were so small as to appear like dots. The central portions of these clumps were quite thick and one received the impression of a granular mass without being able to trace the individual forms composing it. Here and there, one could make out one of the longer spirilla and these could be well seen at the periphery. Some of the clumps were quite small, consisting of only a few spirilla and commas.

Scattered through the stained specimens were many single organisms. The smallest forms seen were slightly curved bacilli or comma forms, a little thickened at the maximum part of the curve. These averaged about 3.4 microns (0.0034 mm.) in length. Rather more common than these were the S-shaped forms which had the appearance of two of the comma forms united. From these one could trace various transitions up to long spirilla measuring as much as 23.5 microns (0.0235 mm.) in length. One could find spirilla of three or four half turns, or two S-shaped forms united by a very delicate thread. Some of the spirilla had as many as sixteen half turns. The ends of these long forms were noticeably pointed and one could make out delicate transparent areas running at right angles to the long axis. The spiral turns were not perfectly regular. Some were quite abrupt. More often they were more gradual and the organisms were slightly bent on themselves. As a rule the spirilla were quite thin and delicate, but there were many forms which were more unevenly stained giving parts of the curves a slightly thickened appearance. No spores could be demonstrated.

August 22, two days before the patient's death, a third culture was taken. All three flasks showed a pure culture of the same organism. The only difference from the previous culture was that the spirilla were recovered from the cultures one day earlier. Following the positive culture, several blood-smears were examined for spirilla with negative results.

SUBCULTURES

Subcultures were taken on various media and the results as given here represent the general average as found after a large number of cultures had been examined.

Plain Broth: In twenty-four hours there is a very slight, diffuse cloudiness with a slight sticky sediment at the bottom of the tube. Stained preparations show many slightly curved bacillary, comma- and S-shaped forms and a moderate number of short spirilla, not exceeding six half turns.

In forty-eight hours the cloudiness becomes more marked. The sediment at the bottom of the tube is much increased in amount and of whitish color. It is quite sticky, and on twirling the tube it forms a long, tree-like, whitish upshoot, remaining attached to the bottom of the tube when its upper end has reached the top. A little more vigorous shaking frees it and breaks it up. Stained smears now show numerous spirilla, many of which are quite long.

On longer incubation the appearance does not change appreciably, the sediment merely showing a moderate increase. Motility continues for as long as three weeks in incubated broth tubes. After this time it gradually dies out.

In broth as in the other cultures, the average time and appearance of the growth is given. When first isolated, the organism took much longer to grow and the growth was much more delicate and sparse.

Glucose Broth: No growth in twenty-four hours. At the end of forty-eight hours, there is a very slight cloudiness in the open arm of the fermentation tube, but none in the closed arm. No gas is formed. Smears show the short forms and a few spirilla of about four half turns. By the end of a week the broth becomes more turbid and there is a little whitish sediment on the sides of the tube which separates out into little sticky threads on shaking. No growth in the closed arm of the tube and no gas.

Lactose Broth: Follows the appearance of the glucose broth in every detail. No gas is formed.

Litmus Milk: Tubes freely inoculated with the organism never showed any change and it was never possible to demonstrate any growth in this media.

Inulin: Not fermented.

Ascitic Glucose Agar: This was the most favorable medium found. However, mediums made from different samples of ascitic fluid did not prove equally favorable. In fact, at times the organism would not grow at all on some of the samples and was finally lost in this way.

At the end of twenty-four hours there are a few minute, semi-transparent, dew-like colonies with a very slight whitish sediment in the water of condensation. Smears at this time show only the small bacillary and comma forms. In forty-eight hours the colonies are slightly larger. Older cultures show a gradual increase in the size of the colonies up to one-half or three-quarters of a cm. in diameter. They are round with a serrated or irregular border. The margins are clean cut. They are distinctly raised above the surface of the medium and are of a dirty white color. With the light shining through, the centers appear thick and brownish, the periphery white and more translucent. Under the low power of the microscope, the margins appear slightly raised. There is a suggestion of radiating lines from the periphery to the center. The sediment in the water of condensation becomes very sticky and rolled up into a little ball-like clump which can be separated out on shaking. The growth on the surface of the medium becomes quite tenacious. The superficial part can be easily removed with the platinum wire, leaving an area of the same size, translucent in appearance which cannot be removed without taking the medium with it.

Glucose Agar: The appearances here are the same as with the ascitic agar, but it takes the colonies about twenty-four hours longer to develop; they are never as numerous, and they do not reach the same size.

Plain Agar: The only difference from the above is that the growth is slower, more uncertain and never as profuse. Many times on this medium the organism fails to grow at all.

Blood Agar: In from twenty-four to forty-eight hours, minute whitish colonies appear, the red of the medium shining through. They are glistening in appearance. Smears show the same picture as in the case of the ascitic agar colonies at the same time. At the end of a week the colonies reach a size of from 3 to 4 mm. in diameter. In appearance these colonies do not differ from those on ascitic agar.

Loeffler's Blood Serum: The growth is generally slow and not profuse. The appearances are the same as with the other solid media.

The organism did not produce indol, did not liquefy gelatin and did not conform to the cultural characteristics of the spirillum of cholera.

Anaerobic Cultures: The organism is a facultative anaerobe. It grows fairly well under anaerobic conditions, appearing as a dirty white, non-transparent cloud in the media. The colonies appear somewhat granular and show a tendency to grow between the column of agar and the side of the tube. When grown under these conditions the organisms are apt to show more regular curves and to occur in longer filaments.

Animal Inoculations: White rats, guinea-pigs, and rabbits were inoculated intraperitoneally and intravenously with 0.5 and 1 c.c. of a twenty-four-hour broth culture without causing any symptoms. At the end of three months these animals were killed and no lesions were found. The organism could not be recovered from the blood at this time. Unfortunately these experiments could not be repeated as the organism died out and was lost.

STAINING

The organism stains well with the ordinary anilin dyes, but the best results are obtained with carbol-fuchsin. It is Gram-negative. Spreads made with India ink show up especially well and the organism can be well studied by means of the dark field illumination.

SUMMARY

We are fully aware that the report of any new organism is always open to the criticism of being a contamination or some secondary infection, having no causal relationship to the disease in question. In spite of this, however, we believe that it is of great importance to report any such organisms where there is apparently just ground for considering them pathogenic. It calls attention to new possibilities and places on record a description of the organism for future reference and comparison.

In the present case, the organism in question seems to be fairly well established as the cause of the disease. The blood-culture results were very satisfactory. On two separate occasions, with about ten days intervening, three broth flasks showed a pure growth of the organism. A third culture, the first one taken, was discarded as sterile on the fifth day. This was two days before the second culture showed a growth and one day before the last culture was positive. It is possible that had it been kept, it, too, would have been positive.

At autopsy, the same organism was obtained from the blood, liver and cardiac vegetations, several times in pure culture, in the other instances in association with the usual autopsy organism, the colon bacillus.

Smears of the vegetations showed the organism very definitely. No other variety of bacteria was seen in these smears. Sections of the vegetations also showed the organism very well and again no other bacteria could be identified.

The animal inoculations were not so satisfactory. They were carried out on only a limited scale and gave only negative results. As mentioned above, these experiments could not be repeated owing to the loss of the organism.

One of the striking things about the case was the fact that the blood-cultures did not become positive until after the time when they are usually discarded. It would seem advisable, in the light of this experience, to keep all blood cultures from unusual cases or those running a temperature for more than five days.

We have been unable to find any reference to an organism similar to the one found in this case and have ventured to give it the name *Spirillum surati*, after the name of the locality in which the disease was presumably contracted.

In conclusion, we wish to express our deep appreciation to Dr. Noguchi for his valuable assistance in the study of the organism and the preparation of this report.

A CASE OF SPIRILLUM INFECTION*

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The following case, while lacking autopsy confirmation, is of considerable interest when considered in conjunction with the case reported in this issue by Drs. Lamb and Paton. Like this, it suggests a form of infection probably overlooked not very infrequently.

Case History.—Male, aged 43, American, widower; occupation, designer. Admitted to Presbyterian Hospital September 23, 1912.

Family History—No bearing.

Past History—Always notably well except for typhoid fever in 1895, and two attacks of malaria in 1911 and July, 1912, respectively, parasites having been found.

Habits—Moderate user of alcohol, heavy user of tobacco. No genito-urinary history obtained.

Present Illness.—Onset sudden on September 2, twenty-one days before admission, with severe nausea, vomiting and inability to retain any food or water. These symptoms continued for five days, at the end of which time the stomach was again apparently normal. On September 20, three days before admission, the same symptoms recurred, this time with moderate jaundice on the second day. Stools were of light color in the beginning, but soon became of normal color. Urine became and continued dark. There was some tenderness under the right costal margin, but no pain radiating from this region. There was, however, sharp pain about the left scapular spine radiating to the left inner arm, also a distinct feeling of oppression about the upper chest.

On admission the physical examination showed a well-developed middle-aged man, quite prostrated, without dyspnea. He was slightly jaundiced. The teeth were in excellent condition, tonsils not enlarged, neck not stiff. The lungs showed a few subcrepitant râles at the right base posteriorly; otherwise they are described as negative. The heart apex impulse was neither seen nor felt, no sounds were to be heard at any point, no adventitious sounds were heard. The area of cardiac dulness was greatly enlarged, extending on the right in the fourth space to 7 cm. to the right of the mid-line, in the fifth space 8 cm. to the right of the mid-line. The left limit of dulness in the third space was 10 cm. to the left of the mid-line; in the fourth space 11.5 cm. to the left of the mid-line; in the fifth space 12 cm. to the left of the mid-line. The pulses were equal and small, their tension seemed low, there was intermission about each fifth to sixth beat. The liver dulness extended up to the fourth space, with flatness from the sixth rib to the costal margin. Below the right costal margin there was a globular mass, slightly tender on palpation, dull, almost flat on percussion. The spleen was not felt. The abdomen was otherwise negative. The extremities were normal.

A tentative diagnosis was made of cholecystitis and pericarditis with effusion. A radiograph taken on the following day revealed the following condition, the report being given verbatim:

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*From the Pathological Laboratory, Presbyterian Hospital, Columbia University, New York.

"Examination not very satisfactory on account of condition of patient; no calculi made out in gall-bladder; heart is very large and dilated; further than this, examination not satisfactory."

Clinical Course.—September 27 there were signs of fluid at the right base posteriorly; the chest was aspirated and about 35 ounces of clear, amber fluid was obtained. An examination of this fluid was as follows:

Quantity, 770 c.c. Specific gravity, 1.011. Sediment, bloody. Differential of 100 cells: Polymorphonuclears, 8 per cent.; lymphocytes, 42 per cent.; endothelial cells, 50 per cent. Sodium chlorid .572, which equals 4.4 gram total. Reaction for bile, positive. No bacteria found in sediment by centrifuge.

September 28 a needle was inserted into the pericardial sac in the fifth space, 11 cm. to the left of the mid-line. A bloody, gelatinous fluid was obtained, showing under the microscope many blood and pus cells and a fair number of spirilla; no other bacteria. This fluid was sterile aerobically on both broth and Loeffler's blood-serum.

At 9 p. m. on the same day, thoracotomy was done and the pericardium drained; 30 c.c. of bloody pus flowed from the pericardial sac. Examination was as follows:

Polymorphonuclears, 96 per cent.; large mononuclears, 1 per cent.; lymphocytes, 1 per cent.; endothelial cells, 2 per cent. Sediment about 7 per cent., consisting roughly of one-third pus and two-thirds blood-cells. Three clear cut spirilla found. No other bacteria. Cultures on glucose agar, ascitic glucose agar and broth aerobically sterile.

September 29, left chest aspirated; 26 ounces of bloody fluid obtained. Smears showed a few spirilla identical with those previously found; no other bacteria; no tubercle bacilli. Cultures, aerobic broth sterile; glucose agar sterile.

October 3, left chest again aspirated and 5 ounces obtained identical with that of September 29.

October 7, tender swelling of the right parotid gland. October 8, the right gland subsiding; the left parotid now somewhat swollen. From this point on the patient weakened steadily until death intervened on October 11.

Five blood-counts made during his stay showed as follows:

On admission:
White blood-cells, 15,000; polymorphonuclears, 73 per cent.

September 30:
White blood-cells, 34,000; polymorphonuclears, 91 per cent.

October 5:
White blood-cells, 15,200; polymorphonuclears, 81 per cent.

October 7:
White blood-cells, 24,400; polymorphonuclears, 84 per cent.

October 10:
White blood-cells, 41,000; polymorphonuclears, 81 per cent.

Eosinophils were never found, nor were any abnormal cells found. The temperature was on admission 103 F.; it fell gradually to normal, reaching that point on the sixth day. It then gradually rose for seven days and finally, during the last week, was of a septic type, but low — 99 to 100 F.

This case was, in the beginning, considered as having its origin below the diaphragm as a process which had extended upward. It was also presumed to be due to some ordinary pyogenic organism. The assumption that the original infection was below the diaphragm was probably correct, but repeatedly sterile cultures and the absence of any organisms, save the spirilla, from the smears make the supposition of a pure

spirillum infection strong. Unfortunately, the condition of the patient was such that it seemed inadvisable to take a blood culture.

DESCRIPTION OF ORGANISM — MORPHOLOGY

The organism is apparently a spirillum, being made up of segments lying end to end. These segments average 0.3 to 0.5 micron in width, and 1 micron in length; their ends are rather abruptly blunt; the waves are regular, rectangular, the wave length averaging 1.2 micron.

Cultures: In the beginning these organisms were found to be anaerobic, no growth being obtained on any of the ordinary culture media aerobically. Anaerobic growth, however, was obtained on the special medium devised by Noguchi for the cultivation of *S. pallida*. After growth on this medium the organism was found to be increasingly aerobic. On this special anaerobic medium there was no coagulation of the protein material and no formation of gas. The odor was a faint musty one, otherwise not unpleasant. The subsequent aerobic growth showed on ascitic agar plates deep colonies which were of a punctate and opaque form. Superficial colonies were about 1 mm. in diameter, of regular outline and of an opaque, grayish white color. In all fluid media, in which there was to a large extent, exclusion of oxygen, by layering with paraffin oil, there was formed a deposit of faint color, in the form of a ring about the tube, between the medium and the overlying oil. As a general rule, the organism was shorter in anaerobic media. The shorter forms, which were often unicellular or composed of only two or three segments, were actively motile, both backward and forward, moving with equal facility and with an extremely rapid lateral vibration. In longer spirilla, made up from eight to twelve organisms, motility was sluggish, rather rigid, with very little flexibility. The motion was of a forward, zig-zag type, the spirilla moving as a rigid whole. The short anaerobic form, grown on the ascitic broth tissue medium, after one week became considerably longer, then appearing as a rule as a spirillum of about the same length as found in the smears of the original pus.

ANIMAL INOCULATIONS

The original pus and cultures were put into the following animals with no apparent effect: Rabbits, intravenously; guinea-pigs, intraperitoneally; rabbits, by injection into the testicle.

For the privilege of reporting this case I am indebted to Dr. George Tuttle. Also I desire to thank Dr. Hideyo Noguchi for very great assistance in the study of the organism.

STUDIES ON SULPHUR METABOLISM. I. THE URINARY SULPHUR PARTITION IN VARIOUS DISEASES *

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I. INTRODUCTION

As is well known, the urinary sulphur is made up of the sulphates, both inorganic and ethereal, and certain less highly oxidized compounds, which are called, following Salkowski's[1] suggestion, "neutral sulphur." The neutral sulphur compounds are many and include the following: uroferric acid,[2] uroproteic acid,[3] oxyproteic acid,[4] urochrome,[5] thiocyanic acid and its salts,[6] cystin and similar substances,[7] taurin and taurin-carbamic acid,[1] methyl mercaptan,[8] ethyl sulphid,[9] thiosulphuric acid[10] and sulphurous acid.[11]

Many of the above mentioned substances contain nitrogen in a form which would be included under the term colloidal nitrogen, and on that account one would expect in those cases in which the urine contains an increased amount of colloidal nitrogen, there would also be an increase in the neutral sulphur of the urine.

It has been known for some time that the colloidal nitrogen of the urine is increased in cancer[12] and there is reason to believe that the

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* From the German Hospital and Dispensary, New York.

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1. Virchows Arch. f. path. Anat., 1873, lviii, 472.

2. Ztschr. physiol. Chem., 1903, xxxvii, 251.

3. Arch. f. exper. Path. u. Pharm., 1897, xl, 29.

4. Centralbl. f. d. med. Wissensch., 1897, p. 577; Ber. d. Deutsch. chem. Gesellsch., 1902, xxxv, 2959; Ztschr. f. physiol. Chem., 1905, xlv, 83; Pflügers Arch. f. d. ges. Physiol., 1899, lxxv, 87.

5. Ztschr. f. physiol. Chem., 1907, liv, 204.

6. Proc. Roy. Soc. London, 1870, xvi, 18; Virchows Arch. f. path. Anat., lxix, 354.

7. Ztschr. f. physiol. Chem., 1885, ix, 125; 1888, xii, 254

8. Arch. f. exper. Path. u. Pharm., 1891, xxviii, 206.

9. Ztschr. f. physiol. Chem., 1894, xx, 253.

10. Ergebn. d. Physiol., 1902, i, 458.

11. Strümpell: Arch. d. Heilk., 1876. (Quoted from Mann, Physiology and Pathology of the Urine, 1908, p. 18.)

12. Töpfer: Wien. klin. Wchnschr., 1892, v, 49; Bondzynski and Gottlieb: Centralbl. f. d. med. Wissensch., 1897, xxxv, 577; Salkowski: Berl. klin. Wchnschr., 1910, xlvii, 1746; Hess and Saxl: Beitr. z. Carcinomforsch., 1910, Part 2; Salkowski and Kojo: Berl. klin. Wchnschr., 1910, xlvii, 2297; Kojo: Ztschr. f. physiol. Chem., 1911, lxxiii, 416; Einhorn, Kahn and Rosenbloom: Am. Jour. Gastro-Enter., 1911, i, 2; Arch. f. Verdauungskr., 1911, xvii, 557; Kahn and Rosenbloom: Biochem. Bull., 1912, ii, 87. Dr. Rosenbloom is studying the relation between "acidosis" and the amount of colloidal nitrogen in the urine.

neutral sulphur of the urine is also increased in cancer. Weiss[13] in a thorough study has found a high increase in the amount of neutral sulphur excreted by patients with carcinoma. His paper also contains a list of all of the neutral sulphur estimations that have been made on urine from patients suffering from various diseases. Salomon and Saxl[14] claim that the urine of carcinomatous individuals contains a neutral sulphur constituent, the sulphur of which can be split off by means of hydrogen peroxid. They found that urine containing this substance yielded 0.01-0.018 grams of $BaSO_4$ from this sulphur per 100 c.c. of urine. Murachi[15] also found this type of sulphur substances present in the urine of cancer patients. He claims it may compose as high as 3.8 per cent. of the total sulphur of the urine and that it belongs to the neutral sulphur fraction. Petersen,[16] in studying this reaction, found it to be positive in case of cancer. Weiss,[13] using the Folin methods of estimation of the sulphur partition of the urine, found in normal men that the neutral sulphur was from 12 to 40 per cent. of the total sulphur, the average being 16.5 per cent. He thinks a part of the neutral sulphur has an exogenous origin, but the greater part is endogenous, and that any condition causing an increased destruction of body protein causes an increased excretion of the neutral sulphur of the urine. He found that there was a marked increase in the neutral sulphur of urine from patients suffering from tuberculosis, while in carcinoma the neutral sulphur was from 20.3 to 36.4 per cent. of the total sulphur. Guitaro[17] found that in normal adult men the neutral sulphur of the urine was 19.41 per cent. of the total sulphur and in normal adult women it was 21.14 per cent. of the total sulphur.

Our work was carried out with the hope that we might be able to find that the increased neutral sulphur of the urine from patients suffering from cancer, would prove a reliable aid in the diagnosis of cancer.

II. METHODS

The patients received the ordinary hospital diet, unless otherwise stated. The urine was collected in twenty-four-hour periods, using powdered thymol as a preservative. The total sulphur of the urine was estimated by Benedict's[18] method, the total and inorganic sulphates by Folin's method.[19] The ethereal sulphates were computed by subtracting the inorganic sulphates from the total sulphates, and the neutral sulphur

13. Weiss: Biochem. Ztschr., 1910, xxvii, 175.
14. Saxl: Deutsch. med. Wchnschr., 1912, xxxviii, 53.
15. Murachi: Biochem. Ztschr., 1912, xli, 138.
16. Petersen: Deutsch. med. Wchnschr., 1912, xxxviii, 1536.
17. Guitaro: Riv. crit. clin. med., 1910, x, 593.
18. Jour. Biol. Chem., 1909, vi, 363.
19. Jour. Biol. Chem., 1905-06, i, 13.

was computed by subtracting the total sulphate-sulphur from the total sulphur. The accompanying tables contain the data obtained in this study.

DISCUSSION OF TABLE 1

In the nine cases of diabetes studied the neutral sulphur was increased with the exception of Case 4. This case was a mild type, the urine being sugar free when the patient was on a carbohydrate free diet. The other cases were of the severe type with the exception of Case 7. It is interesting to note that the colloidal nitrogen of the urine has been found to be increased also in cases of diabetes.[20] In this series of estimations the average daily total sulphur excretion is 0.94 gm., the average neutral sulphur is 0.21 gm., or 22.3 per cent. of the total sulphur.

DISCUSSION OF TABLE 2

It may be noted that in the thirteen cases of cancer the neutral sulphur of the urine is consistently high both in amount and in proportion to the total sulphur of the urine, with the exception of Case 18, in which the neutral sulphur is not increased.[21] In these cases of carcinoma the lowest neutral sulphur excretion is 0.05 gm., and the highest 0.76 gm. In relation to the proportion of total sulphur excreted as neutral sulphur, the lowest is 6.2 per cent. and the highest 67.9 per cent. While the average total sulphur excreted is 0.88 gm., the average neutral sulphur is 0.20 gm. or 23.1 per cent. of the total sulphur.

DISCUSSION OF TABLE 3

A marked increase in the neutral sulphur of the urine was found in one case of interstitial nephritis, in one case of lobar pneumonia and in one case of hypopituitarism. In one case of interstitial nephritis and in one case of pneumonia it was not increased. Very low neutral sulphur excretions were found in two cases of chronic lead poisoning. In two cases of chronic appendicitis there was no increase. In one case of bronchial asthma there was a slight increase. In one case of hepatic abscess and in one case of syphilitic hepatitis there was no increase. In one case of perihepatitis and in one case of cholelithiasis there was an increase of the neutral sulphur of the urine, only when bile was excreted in the urine. In one case of typhoid fever, of chronic myocarditis with broken compensation, and one case of gastroptosis and gastric dilatation, there was no increased neutral sulphur excretion.

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20. Einhorn, Kahn and Rosenbloom: Am. Jour. Gastro-Enter., 1911, i, 2; Archiv. f. Verdauungskr., 1911, xvii, 557.

21. It is to be remembered that this case clinically resembled carcinoma of the stomach. It is possible, however, that the diagnosis was wrong. Unfortunately we were not able to follow the case to operation.

TABLE 1.—THE URINARY SULPHUR PARTITION IN DIABETES MELLITUS

Case No.	Date.	Total Sulphur, gm.	Sulphate Sulphur, gm.	Ethereal Sulphate Sulphur, gm.	Inorganic Sulphate Sulphur, gm.	Neutral Sulphur, gm.	Sulphate Sulphur ÷ Total Sulphur, Pct.	Ethereal Sulphate C. ÷ Total Sulphur, Pct.	Inorg. Sulphate S. ÷ Total Sulphur, Pct.	Neutral Sulphur ÷ Total Sulphur, Pct.	Diagnosis and Remarks.
1	6/27	1.04	0.89	0.059	0.829	0.151	85.5	5.6	79.9	14.5	Diabetes mellitus with gangrene of toe; patient on strict antidiabetic diet. Urine still contains sugar.
1	7/6	1.52	0.98	0.088	0.894	0.538	64.8	5.8	59.0	35.4	
1	7/7	1.16	0.83	0.079	0.749	0.332	71.4	6.8	64.6	28.6	
1	7/8	0.91	0.77	0.087	0.684	0.139	84.7	9.5	75.2	15.2	
1	7/9	1.20	1.07	0.045	1.025	0.130	89.1	3.7	85.4	10.8	
1	7/10	1.98	1.85	0.151	1.699	0.130	93.4	7.6	85.8	6.5	
1	7/11	1.31	1.15	0.027	1.123	0.160	87.7	2.1	85.6	12.2	
1	7/12	1.30	1.15	0.089	1.061	0.150	88.4	6.8	81.6	11.5	
1	7/13	1.01	0.86	0.087	0.773	0.150	85.1	8.6	76.5	14.8	
2	7/8	0.893	0.635	0.055	0.580	0.258	71.1	6.2	64.9	28.8	Diabetes mellitus. Strict anti-diabetic diet. Urine still contains sugar.
2	7/9	0.871	0.519	0.028	0.491	0.352	59.6	3.2	56.4	40.4	
2	7/10	0.583	0.470	0.016	0.454	0.093	86.2	2.8	83.4	15.9	
2	7/12	1.320	0.963	0.014	0.949	0.357	72.9	1.1	71.8	27.1	
2	7/13	0.737	0.604	0.014	0.590	0.133	81.9	1.9	80.0	18.1	
2	7/14	0.589	0.475	0.014	0.461	0.114	80.8	2.4	78.4	19.4	
2	7/15	0.750	0.623	0.013	0.610	0.127	83.1	1.7	81.4	16.9	
3	6/27	1.61	1.216	0.073	1.143	0.394	75.5	4.53	70.97	24.4	Diabetes mellitus with gangrene of foot. Strict anti-diabetic diet. Urine still contains sugar.
3	6/28	0.872	0.626	0.092	0.614	0.246	71.8	10.6	61.2	28.2	
3	7/6	1.686	1.20	0.091	1.109	0.486	71.1	5.4	65 7	28.8	
3	7/7	0.369	0.226	0.027	0.199	0.143	61.3	7.3	54.0	38.8	
3	7/8	0.454	0.241	0.051	0.190	0.213	53.1	11.2	41.9	46.9	
3	7/9	1.12	0.865	0.013	0.852	0.255	77.2	1.16	76.04	22.8	
3	7/10	1.20	0.989	0.098	0.891	0.211	82.4	8.1	74.3	17.5	
3	7/11	0.554	0.287	0.002	0.285	0.267	51.8	3.6	48.2	48.2	
3	7/12	1.404	1.09	0.061	1.029	0.314	77.6	4.3	73.3	22.4	
4	7/6	0.84	0.79	0.03	0.76	0.05	94.0	3.6	90.4	5.9	Diabetes mellitus with gangrene of foot. Strict anti-diabetic diet. Urine is free from sugar.
4	7/8	1.12	1.05	0.016	1.034	0.07	93.8	1.4	92.4	6.2	
4	7/9	0.666	0.61	0.065	0.545	0.056	91.6	9.8	81.8	8.4	
4	7/10	0.881	0.835	0.073	0.762	0.046	94.8	8.3	86.5	5.2	
4	7/13	0.92	0.823	0.072	0.751	0.097	89.5	7.8	81.7	10.5	
4	7/15	0.41	0.36	0.068	0.792	0.05	87.8	16.6	71.2	12.2	
5	7/2	0.822	0.689	0.086	0.603	0.133	83.8	10.4	73.4	16.1	Diabetes mellitus with gangrene of foot. Strict anti-diabetic diet. Urine contains sugar.
5	7/7	0.751	0.621	0.013	0.612	0.128	82.7	1.73	81.2	15.7	
6	6/28	1.09	0.851	0.116	0.735	0.239	78.1	10.7	67.4	21.9	Diabetes mellitus. Strict anti-diabetic diet. Urine contains sugar.
6	7/1	1.34	0.936	0.088	0.848	0.404	69.8	6.5	63.3	30.1	
6	7/22	0.38	0.247	0.018	0.229	0.130	65.5	4.8	60.7	34.5	
6	7/28	0.693	0.555	0.032	0.52	0.138	80.05	4.67	75.4	19.9	
7	6/25	1.97	1.244	0.121	1.123	0.726	63.1	6.1	57.0	36.8	Diabetes mellitus. Fifty gm. bread tolerance. Urine free from sugar.
7	7/2	1.395	1.225	0.116	1.109	0.170	87.8	8.3	79.5	12.2	
8	6/29	0.924	0.728	0.099	0.629	0.196	78.8	10.7	68.1	21.2	Diabetes mellitus. Strict anti-diabetic diet. Urine free from sugar.
8	7/1	0.866	0.694	0.114	0.58	0.172	80.1	13.2	66.9	19.8	
8	7/3	0.921	0.721	0.09	0.631	0.20	78.3	9.8	68.5	21.7	125 gm. of bread added to diet. Urine contains sugar.
8	7/5	1.06	0.83	0.011	0.819	0.23	78.3	10.4	67.9	21.7	
8	7/6	0.945	0.81	0.116	0.694	0.135	85.7	12.3	73.4	14.3	100 gm. of bread added to diet. Urine contains sugar.
8	7/7	0.925	0.598	0.076	0.522	0.327	64.7	8.2	56.5	35.3	75 gm. of bread added to diet. Urine free of sugar.
8	7/8	0.925	0.739	0.099	0.64	0.186	79.9	10.7	69.2	20.1	75 gm. of bread added to diet. Urine free of sugar.
8	7/9	0.98	0.752	0.084	0.668	0.227	76.8	8.6	68.2	23.2	100 gm. of bread added to diet. Urine contains sugar.
9	7/6	0.404	0.275	0.021	0.254	0.129	68.1	5.2	62.9	31.9	Diabetes mellitus. Strict anti-diabetic diet. Urine contains sugar.
9	7/9	0.331	0.264	0.031	0.233	0.067	79.8	9.4	70.4	20.3	
9	7/16	0.326	0.289	0.03	0.259	0.037	88.7	9.2	79 5	11.3	
9	7/18	0.372	0.30	0.03	0.27	0.072	80.7	8 7	72.0	19.3	
9	7/19	0.271	0.20	0.034	0.166	0.07	73.8	12.5	61.3	25.8	
9	7/26	0.192	0.161	0.038	0.124	0.031	83.8	19.5	64.3	16.1	

TABLE 2.—THE URINARY SULPHUR PARTITION IN CANCER

Case No.	Date.	Total Sulphur, gm.	Sulphate Sulphur, gm.	Ethereal Sulphate Sulphur, gm.	Inorganic Sulphate Sulphur, gm.	Neutral Sulphur, gm.	Sulphate Sulphur ÷ Total Sulphur, Pct.	Ethereal Sulphate S. ÷ Total Sulphur, Pct.	Inorg. Sulphate S. ÷ Total Sulphur, Pct.	Neural Sulphur ÷ Total Sulphur, Pct.	Diagnosis and Remarks.
11	7/3	0.78	0.25	0.09	0.16	0.53	32.1	11.1	21.0	67.9	Carcinoma of cervix. Phlegmon of abdomen.
12	6/30	0.83	0.61	0.06	0.55	0.22	73.9	6.9	67.0	26.0	Carcinoma of rectum.
13	6/27	1.33	1.06	0.097	0.96	0.27	79.7	7.3	72.4	20.3	Carcinoma of stomach.
13	6/28	2.84	2.19	0.095	2.09	0.65	77.1	3.3	73.8	22.9	
14	7/29	0.50	0.34	0.05	0.297	0.155	69.0	9.6	59.4	31.0	Carcinoma of lung. Metastatic from breast.
15	7/26	0.78	0.65	0.037	0.613	0.13	83.3	4.7	78.6	16.6	Carcinoma of cervix.
15	7/27	0.82	0.68	0.073	0.614	0.14	82.9	8.9	74.0	17.1	Patient on Folin standard
15	7/28	0.60	0.50	0.11	0.39	0.12	82.7	18.3	64.4	20.0	diet.
15	7/29	0.62	0.50	0.11	0.39	0.12	80.7	17.7	63.0	19.4	
15	7/30	0.78	0.65	0.037	0.613	0.13	83.3	4.7	78.6	16.6	
16	7/8	0.60	0.49	0.047	0.446	0.11	81.7	7.7	74.0	18.2	Carcinoma of breast and
16	7/21	0.62	0.51	0.049	0.449	0.11	81.7	7.6	73.9	18.2	pulmonary tuberculosus.
17	7/7	0.24	0.13	0.018	0.109	0.11	53.8	7.6	46.2	46.2	Cystadenoma of ovary with peritoneal metastases.
18	7/25	0.39	0.34	0.048	0.292	0.05	87.2	12.3	74.9	12.8	Carcinoma of stomach.
18	7/26	0.58	0.51	0.047	0.463	0.07	87.9	8.1	79.8	12.1	
18	7/27	0.55	0.49	0.06	0.42	0.06	89.1	10.9	78.2	10.9	
18	8/9	0.78	0.68	0.096	0.58	0.10	87.2	12.3	74.9	12.8	
18	8/15	1.08	0.94	0.11	0.83	0.137	87.3	10.3	77.0	12.6	
18	8/17	0.728	0.64	.....	.....	0.086	88.2	.....	.....	11.7	
18	8619	0.74	0.62	.....	.....	0.12	83.6	.....	.....	16.3	
18	8/22	0.67	0.61	.....	.....	0.058	91.3	.....	.....	8.6	
18	8623	0.77	0.72	.....	.....	0.048	93.7	.....	.....	6.2	
18	8/25	1.02	0.89	.....	.....	0.125	87.6	.....	.....	12.3	
18	8/26	0.348	0.284	.....	.....	0.063	84.6	.....	.....	18.5	
18	8/27	0.616	0.534	.....	.....	0.121	86.8	.....	.....	19.6	
18	8/28	0.74	0.62	.....	.....	0.120	83.6	.....	.....	16.3	
18	8/29	0.92	0.76	.....	.....	0.16	82.9	.....	.....	17.0	
18	8/30	1.02	0.89	.....	.....	0.125	87.6	.....	.....	12.3	
19	6/21	0.995	0.753	0.101	0.65	0.24	75.6	10.1	65.5	24.4	Early carcinoma of larynx.
19	6/22	0.712	0.47	0.127	0.34	0.24	65.9	17.8	48.1	34.0	
19	6/28	1.82	1.2	0.183	0.68	0.62	65.9	10.0	55.9	34.0	
19	6/29	1.86	1.47	0.111	1.36	0.39	79.1	5.9	73.2	20.9	
19	6/30	1.44	0.67	0.111	0.56	0.76	46.8	7.7	39.1	53.2	
19	7/3	0.62	0.42	0.119	0.30	0.20	67.6	19.1	48.5	32.4	
20	8/9	0.86	0.78	0.053	.....	0.17	91.6	6.1	.....	20.0	Carcinoma of glands of neck. Operated two months ago.
21	8/9	0.61	0.50	0.04	0.46	0.11	81.9	6.5	75.4	18.1	Carcinoma of larynx and
21	8/12	0.90	0.63	0.037	.....	0.262	70.8	4.0	.....	29.1	esophagus. Gastrostomy
21	8/14	0.49	0.41	0.032	.....	0.081	83.4	6.5	.....	16.5	on July 29.
21	8/15	0.78	0.58	0.052	.....	0.26	73.5	6.2	.....	32.8	
21	8/16	0.63	0.53	0.04	.....	0.113	83.6	6.5	.....	18.0	
22	6/22	1.02	0.716	0.099	0.617	0.304	70.2	9.7	60.5	29.8	Inoperable carcinoma of
22	6/23	0.456	0.354	0.058	0.296	0.102	77.6	12.7	64.9	22.4	stomach.
22	6/27	0.81	0.61	0.123	0.486	0.198	75.4	15.2	60.2	24.5	
22	7/3	0.41	0.308	0.089	0.015	0.098	75.8	22.1	53.7	24.1	
23	6/22	0.898	0.70	0.124	0.577	0.197	78.1	13.8	64.3	21.9	Inoperable carcinoma of
23	6/23	0.814	0.587	0.052	0.535	0.227	72.1	6.4	65.7	27.9	stomach.
23	6/30	1.64	1.28	0.084	1.196	0.362	77.9	5.1	72.8	22.1	
23	7/1	2.17	1.53	0.122	0.812	0.639	70.6	5.6	65.0	29.4	

TABLE 3.—The Urinary Sulphur Partition in Various Other Diseases

Case No.	Date.	Total Sulphur, gm.	Sulphate Sulphur, gm.	Ethereal Sulphate Sulphur, gm.	Inorganic Sulphate Sulphur, gm.	Neutral Sulphur, gm.	Sulphate Sulphur ÷ Total Sulphur, Pet.	Neutral Sulphur ÷ Total Sulphur, Pet.	Ethereal Sulphate S. ÷ Total Sulphur, Pet.	Inorg. Sulphate S. ÷ Total Sulphur, Pet.	Diagnosis and Remarks.
24	6/30	1.13	0.33	0.08	0.25	0.80	29.1	6.9	22.2	70.8	Chronic interstitial nephritis. Mag. sulph. given.
25	7/24	0.812	0.68	0.06	0.62	0.132	83.7	7.3	76.4	16.2	Chronic interstitial nephritis.
26	8/8	0.66	0.56	0.059	0.50	0.095	84.7	9.5	76.2	14.4	Fracture of ribs with pneumonia.
26	8/10	0.63	0.54	0.032	0.51	0.09	85.2	5.0	80.2	14.7	
26	8/12	0.74	0.54	0.04	0.50	0.198	73.1	55.7	67.6	26.8	
26	8/18	0.86	0.72	.....	.....	0.136	83.7	.....	.....	15.7	
26	8/20	0.87	0.73	0.047	0.68	0.136	83.9	5.4	78.5	15.8	
26	8/25	0.77	0.73	.....	.....	0.04	94.8	.....	.....	5.3	
27	7/29	1.98	0.17	0.078	0.923	1.816	8.5	3.9	4.6	90.1	Lobar pneumonia.
28	8/14	1.13	1.10	0.057	1.04	0.028	97.5	5.2	92.4	2.4	Chronic lead poisoning. Mag. sulph. and potassium iodid treatment.
28	8/16	1.46	1.37	0.053	1.31	0.089	93.8	3.6	90.1	4.0	
28	8/17	1.22	1.07	0.03	1.06	0.150	87.4	2.5	86.9	12.5	
29	8/4	0.95	0.88	0.025	0.925	0.121	92.5	2.6	97.3	12.7	Chronic lead poisoning. Mag. sulph. and potass. iodid treatment.
29	8/5	1.17	1.09	0.024	1.124	0.08	93.1	20.5	96.2	7.1	
30	6/27	0.74	0.53	0.05	0.47	0.22	70.9	7.3	63.6	29.1	Bronchial asthma. Epinephrin and KI given.
31	7/5	0.28	0.22	0.02	0.20	0.06	77.5	7.1	70.4	22.5	Chronic appendicitis one week after operation.
32	7/8	0.395	0.355	0.043	0.312	0.04	89.9	10.9	79.0	10.1	Clinically gastric-carcinoma; operation showed chronic appendix.
32	7/10	0.248	0.216	0.036	0.18	0.03	87.1	14.5	72.6	12.9	
33	7/26	0.552	0.44	0.05	0.39	0.11	79.7	9.1	70.6	19.9	Hepatic abscess.
34	7/25	0.60	0.50	0.11	0.39	0.12	82.7	18.3	64.4	20.0	Syphilitic hepatitis. Carlsbad salts given.
34	7/26	1.01	0.86	0.094	0.766	0.15	85.1	9.3	75.8	14.8	
35	6/21	3.36	1.19	0.143	1.05	2.17	35.4	4.3	31.1	64.6	Perihepatitis at operation. Bile in urine. Carlsbad salts daily. Urine free from bile.
35	6/22	1.37	1.13	0.094	1.04	0.24	82.5	6.9	75.6	17.5	
36	6/28	2.86	1.84	0.12	0.73	1.02	64.4	4.2	60.2	35.5	Cholelithiasis with biliary fistula. (Slow in healing.) During period 6/28 to 7/6 the urine contained bile.
36	7/3	2.80	1.89	0.12	0.87	0.91	67.5	4.2	63.3	32.5	
36	7/5	1.61	1.08	0.134	0.94	0.53	67.0	8.3	58.7	33.1	
36	7/6	1.69	1.25	0.163	1.08	0.44	73.8	9.7	64.1	26.1	
36	7/7	1.13	1.07	0.12	0.95	0.06	94.7	10.6	84.1	5.3	
36	7/8	1.48	1.34	0.11	1.23	0.14	90.6	7.3	83.3	9.4	Urine free from bile.
36	7/9	1.28	1.09	0.09	1.00	0.19	85.2	6.8	78.4	14.8	
36	7/10	1.50	1.31	0.08	1.23	0.19	87.4	5.3	82.1	12.8	
36	7/12	1.55	1.38	0.16	1.22	0.17	89.0	10.4	78.6	10.9	
36	7/13	0.72	0.49	0.11	0.37	0.23	67.4	15.7	51.7	32.6	Urine again contained bile.
36	7/14	2.80	1.88	0.12	0.86	0.91	67.5	4.2	63.3	32.5	
37	8/17	0.68	0.60	0.033	0.57	0.08	87.9	4.8	83.1	10.6	Typhoid fever. Second week of disease.
37	8/22	0.86	0.72	0.033	0.68	0.147	83.0	3.8	79.2	16.9	
37	8/24	0.62	0.54	.....	.....	0.08	87.0	.....	.....	12.9	
37	8/25	0.49	0.41	.....	.....	0.08	82.9	.....	.....	17.03	
37	8/28	0.58	0.48	.....	.....	0.10	82.7	.....	.....	17.3	
38	7/15	0.49	0.43	0.048	0.085	0.057	88.4	9.8	78.6	11.7	Chronic myocarditis with broken compensation. Codein given.
38	7/25	0.48	0.42	0.034	0.086	0.060	87.5	7.1	80.4	12.5	
38	7/27	0.37	0.35	0.090	0.026	0.020	94.6	24.3	70.3	5.4	
39	6/23	0.57	0.45	0.04	0.41	0.12	78.6	6.8	71.8	21.3	Hypopituitarism. Patient on standard Folin diet.
39	6/26	0.41	0.30	0.03	0.27	0.11	72.5	7.2	65.3	27.4	
39	6/27	0.80	0.66	0.06	0.60	0.14	82.4	7.1	75.3	17.5	
39	6/28	0.90	0.69	0.04	0.65	0.21	76.7	4.3	72.4	23.3	
39	6/29	0.87	0.53	0.05	0.48	0.33	61.7	6.4	55.3	38.3	
39	6/30	0.96	0.67	0.04	0.63	0.30	69.2	4.0	65.2	30.8	
40	7/26	0.38	0.32	0.024	0.35	0.06	96.3	6.3	90.0	15.8	Gastroptosis and gastric dilatation.
40	7/27	0.76	0.60	0.068	0.61	0.160	78.9	8.9	70.9	21.0	
40	7/28	0.79	0.67	0.059	0.61	0.120	84.8	7.4	77.4	15.2	

In this series, consisting of seventeen cases of various diseases other than carcinoma and diabetes, with fifty-one individual estimations, the daily average excretion of total sulphur is 0.96 gm., of neutral sulphur 0.33 gm., or 34.3 per cent. of the total sulphur. If we exclude from this group a case of lobar pneumonia with an unusually high amount of total and neutral sulphur, we still find the average total sulphur excretion to be .090 gm. and the average neutral sulphur 0.24 gm., or 26.7 per cent. of the total sulphur.

III. CONCLUSIONS

1. The lowest average total sulphur excretion (0.88 gm. per day) was found in a series of thirteen cases of carcinoma.

2. The same series showed also the lowest average neutral sulphur excretion (0.20 gm. per day).

3. The proportion of the neutral sulphur to the total sulphur in this group is considerably higher than the normal proportion of the total sulphur excreted as neutral sulphur.

4. However, the relation of the neutral sulphur to the total sulphur is still higher in the group of seventeen various diseases (not including diabetes or carcinoma). In this group we find that both the relative and absolute amounts of total sulphur and neutral sulphur to be higher than in cancer and diabetes.

5. Eight out of nine cases of diabetes studied showed an increased excretion of neutral sulphur, both in amount and in relation to the total sulphur excreted.

6. From our experience we think it is a precarious undertaking to diagnose a malignant tumor on the basis of the absolute or relative amount of the neutral sulphur excreted in the urine, or from the daily excretion of total sulphur.

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THE REACTION OF SALOMON AND SAXL AS A DIAGNOSTIC TEST FOR CARCINOMA *

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Salomon and Saxl[1] have described a reaction in the urine which they consider to be characteristic of carcinoma. The reaction depends on the supposed presence in the urine of such patients of a sulphur-containing substance, which does not yield inorganic sulphate on treatment with dilute hydrochloric acid, but which, on subsequent treatment with hydrogen peroxid, is oxidized, with liberation of inorganic sulphate. They report positive results in 70 per cent. of cases of carcinoma. They state that the reaction is frequently positive in the urine of pregnant women and in the urine of patients with cirrhosis or abscess of the liver. In a second paper[2] they describe the following modification of their previous method:

> One hundred c.c. of the albumin-free urine are measured into a 400 to 500 c.c. beaker, 10 c.c. of hydrochloric acid (spec. grav. 1.12) are added, the contents of the beaker are heated to boiling on an asbestos-center wire-gauze and 200 c.c. of boiling water are added at once. If the specific gravity of the urine is less than 1.020, 10 c.c., if greater than 1.020, 15 c.c. of 10 per cent. barium chlorid solution are added, drop by drop. The beaker is covered with a watch-glass and kept on a boiling water-bath for six hours. After standing twenty-four hours the liquid is filtered through a double filter and refiltered into a 500 c.c. Erlenmeyer flask. Three c.c. of perhydrol (Merck's 30 per cent H_2O_2) are added and the mixture is boiled for fifteen minutes. It is then transferred to a conical vessel and allowed to stand. The deposit of a perceptible precipitate within a few hours is regarded as a positive reaction.

According to Kaldeck,[3] the reaction is not characteristic of carcinoma. He found that in the urines of nine patients with carcinoma, the reaction was positive in four, negative in four and doubtful in one. Of thirty-seven urines from other patients, eight gave positive results. Of the eight, five were from tuberculous patients, out of a total of nine examined. Pribram[4] tested the urine of forty patients with carcinoma, five with sarcoma and forty who were free from malignant growths. Of the cancer urines, 60 per cent. gave positive reactions; of the others, only 35 per cent. He recommended the use of potassium permanganate as the

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* From the Chemical Laboratory of the Montefiore Home.

1. Salomon and Saxl: Wien. klin. Wchnschr., 1911, xiii, 449.
2. Salomon and Saxl: Deutsch. med. Wchnschr., 1912, xxxviii, 53.
3. Kaldeck: Wien. med. Wchnschr., 1911, lxi, 1652.
4. Pribram: Wien. klin. Wchnschr., 1911, xxiv, 1235.

oxidizing agent because it yielded a clearer liquid in which the precipitate could more readily be detected. Murachi[5] weighed the barium sulphate precipitated in the test. He had urines from four patients with carcinoma and from sixteen other patients. In the former, the amount of barium sulphate obtained varied from 0.0080 to 0.0176 gm., averaging 0.0120 gm., in 100 c.c. of urine, or 0.0779 in the twenty-four-hour quantity, or 2.43 per cent. of the total sulphur. The other urines yielded from 0.0020 to 0.0080 gm., averaging 0.00485 gm. in 100 c.c., or 0.0560 gm. in the twenty-four-hour quantity, or 1 per cent. of the total sulphur. Very good results have been reported by Petersen.[6] Only three out of fifty-five urine patients without carcinoma gave the reaction, whereas

TABLE 1.—NORMAL URINES

Name	$BaSO_4$ from 100 c.c.		$BaSO_4$ from Twenty-Four Hour Quantity		Ratio of Sulphur Precipitated in the Test to Total Sulphur, Per Cent.
	In the Test, mg.	Total Sulphur, gm.	In the Test, gm.	Total Sulphur, gm.	
I. G. ...	11.0	936	.....	.....	1.18
I. G. ...	7.8	811	.....	.....	0.96
I. G. ...	9.8	798	0.127	10.350	1.23
W. R. ..	5.3	333	.....	.....	1.60
B. G. ...	15.0	1001	0.078	5.205	1.50
B. G. ...	16.7	1107	0.149	9.982	1.50
J. B. ...	6.8	666	0.105	10.320	1.02
P. K. ...	6.5	692	0.091	9.688	0.94
M. W. ..	6.7	670	0.061	6.131	1.00

of nineteen urines from seventeen cases of carcinoma only two failed to do so. The patients from whom these two urines were obtained were cachectic, and Petersen believes that this may be responsible for the negative results in these cases. He does not attempt to explain how cachexia operates to cause the disappearance of a reaction supposed to be characteristic of carcinoma.

There seems to be a general agreement among these writers that the reaction, although not characteristic of carcinoma, is much more frequent in the urines of patients with carcinoma than in other urines. It should be remembered, however, that Murachi was the only one who regularly weighed the barium sulphate obtained, and that he had only four cases of carcinoma. This is very important, for a precipitate is obtained with any urine, and normal and carcinoma urines are said to differ only

5. Murachi: Biochem. Ztschr., 1912, xli, 139.
6. Petersen: Deutsch. med. Wchnschr., 1912, xxxviii, 1536.

in the amount of precipitate obtained. It is very difficult, indeed, to compare the amounts of barium sulphate produced under the conditions of the test without actually weighing them, for the absorbed organic material varies greatly in amount and character, but is always considerable.

At the request of Dr. S. Wachsmann, medical director of this institution, I tried this reaction in a number of urines. It soon became evident that the differences that might exist between urines from patients with and without carcinoma were too slight to be detected by simple inspection. Thereafter the precipitates obtained were weighed. The

TABLE 2.—VARIOUS PATHOLOGICAL URINES

Name	Diagnosis	$BaSO_4$ from c.c		$BaSO_4$ from Twenty-Four Hour Quantity		Ratio of Sulphur Precipitated in the Test to Total Sulphur, Per Cent.
		In the Test, mg.	Total Sulphur, mg.	In the Test, gm.	Total Sulphur, gm.	
H. B.	Nephritis, myocarditis	3.0	292	0.051	4.964	1.03
A. S.	Muscular atrophy....	5.5	693	0.039	4.751	0.80
W.	Levulosuria...	4.2	388	0.067	6.208	1.09
L. M.	Cirrhosis of liver	12.6	840	0.064	4.284	1.50
E. S.	Myelogenous leukemia...	7.4	321	0.109	4.719	2.31
M. R.	Diabetes.....	3.5	342	0.034	3.320	1.01
S.	Pulmonary tuberculosis.	3.5	438	0.049	6.139	0.80
	Pneumonia.	9.2	770	0.149	12.470	1.20

directions of Salomon and Saxl were carefully followed, the only deviations being the following:

1. A single thickness of Schleicher and Schull's No. 589 blue ribbon filter paper was used to filter the liquid after heating with acid and standing a day. The filtrates were clear.

2. After treatment with perhydrol the mixture was allowed to stand overnight and was then filtered through a Gooch filter. The precipitate and filter were washed thoroughly, ignited and weighed.

Total sulphur was determined by Benedict's method.[7] Most of the determinations, both of the barium sulphate precipitated by the procedure of Salomon and Saxl and of the barium sulphate obtained from the total sulphur were made in duplicate.

7. Benedict: Jour. Biol. Chem., 1909, vi, 363.

The figures given in the accompanying tables require little comment. The amount of barium sulphate precipitated in the test from normal and pathological urines varies greatly. The highest value was obtained in a normal urine and the lowest in a urine from a patient with a very extensive carcinoma, primary in an ovary. There is apparently no relation between the amount of barium sulphate precipitated in this test and the presence or absence of carcinoma.

TABLE 3.—URINES FROM CASES OF MALIGNANT NEOPLASMS

Name	Diagnosis		$BaSO_4$ from 100 c.c.		$BaSO_4$ from Twenty-Four Hour Quantity		Ratio of Sulphur Precipitated in the Test to Total Sulphur, Per Cent.
	Carcinoma of	Established at	In the Test, mg.	Total Sulphur, mg.	In the Test, gm.	Total Sulphur gm.	
S. B.	Stomach	Operation.....	4.4	260	0.055	3.221	1.69
S. B.	Stomach	Operation.....	3.4	182	0.049	2.621	1.87
R. R.	Ovaries	Autopsy.....	0.7	182	0.011	2.957	0.38
Sol.	Stomach	Operation.....	10.0	607	0.042	2.549	1.64
G. P.	Ovaries	Operation.....	3.5	417	0.041	4.800	0.86
W. F.	Larynx	Operation.....	7.0	591	.....	.....	1.18
S. R.	Stomach and colon	Clinical examination	11.2	710	.....	.....	1.58
Spiv.	Peritoneal cavity	Operation.....	16.5	678	0.201	8.272	2.43
Leit.	Breast	Operation.....	9.8	611	0.073	4.591	1.59
S. H.	Esophagus ...	Autopsy.....	7.1	361	0.069	3.500	1.94
I. G.	Mastoid	Operation.....	9.1	760	0.066	5.470	1.20
E. G.	Larynx	Operation.....	6.7	650	0.088	7.540	1.03
	Sarcoma of						
L. P.	Lungs	Clinical examination	5.8	489	0.037	3.130	1.19
F. R.	Hip	Clinical examination	9.6	753	.....	.....	1.28
H. B.	Peritoneal cavity	Operation.....	6.5	636	0.066	6.420	1.02
M. A.	Thigh	X-Ray examination	9.2	297	0.078	2.520	3.10

It is true that most of the carcinoma patients were cachectic. It may be urged that this explains the discrepancy between the results here reported and those obtained by others, but this is not probable. Several urines were obtained from non-cachectic carcinoma patients. The amounts of barium sulphate obtained from these urines were not greater than those obtained from many of the normal urines. It would also be rather remarkable that the writers mentioned had obtained urines from patients with carcinoma, only comparatively few of whom were cachectic. Moreover, the value of a proposed test for carcinoma which is negative

in cachexia, one of the most characteristic symptoms of the disease, is, on this ground alone, open to serious question.

SUMMARY

No differences were found to exist between the urines of patients with carcinoma and other diseases and normal individuals, in the amount of barium sulphate, either absolute or relative to the total sulphur, precipitated by the procedure of Salomon and Saxl. It is therefore concluded that the test is of no value in the diagnosis of carcinoma.

FURTHER OBSERVATIONS ON THE PROTEIN METABOLISM OF NORMAL PREGNANCY *

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A few months ago we reported in preliminary form[1] the results of complete nitrogen partition studies on three carefully controlled normal cases and five pathological cases of pregnancy. We found that some instances of high ammonia could only be explained on the assumption that the bladder had been contaminated by the use of the catheter, and in one case of eclampsia we were able to demonstrate a marked fall in this constituent of the urine by the simple expedient of thoroughly washing the bladder with boric-acid solution twice in twenty-four hours. Because of the obvious importance of this source of error in judging the significance of such findings, we determined to follow up the practice of washing the bladder whenever a high amount of ammonia was encountered. In all properly conducted metabolism studies on the dog, the twenty-four-hour urines are obtained by catheter, by washing out the residual urine with sterile water and then following this with a wash of saturated solution of boric acid. It is self-evident that we should not be content with a less perfect technic in collecting urines from bed-ridden patients, if the analysis of the urine is to be trustworthy. We have, accordingly, selected a number of normal cases, some of which showed an amount of ammonia above the normal, some of them a normal amount, and placing them on constant diets, have observed the effects of a careful irrigation with a quart of boric-acid solution once or twice within an experiment day.

THE COURSE OF THE AMMONIA OUTPUT IN NORMAL PREGNANCY

We shall present first the urinary findings of two patients who ran perfectly normal courses just before, through and just following labor, the irrigation of whose bladders caused no alteration in the ammonia findings. Such a result can only mean that the bladder was sterile to begin with, and that the use of the catheter introduced no organism capable of splitting urea. The two cases were under the constant sur-

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* Submitted for publication June 13, 1913.

* All of the cases here reported were patients in the maternity wards, second division Bellevue Hospital on the service of Dr. J. Clifton Edgar.

1. Murlin and Bailey: Protein Metabolism in Late Pregnancy and the Puerperium, Jour. Am. Med. Assn., 1912, lix, 1522.

veillance of a special nurse and were maintained on a diet of milk, eggs, bread and butter, potato and ice cream, containing, as shown by the tables, an approximately uniform amount of gross potential energy. The number of calories per kilogram in the tables rises after delivery for the obvious reason that the weight was contantly decreasing. The food was not analyzed, but it is apparent from the total nitrogen figures on the days when a complete twenty-four-hour urine was obtained, that the variation in nitrogen content was not very great. The influence of the total intake of protein and energy, therefore, was reasonably constant throughout.

Before considering the tables, we wish to emphasize the fact that our determinations of the ammonia in these and all other cases have been made by the most approved technic of the Folin method. Folin has recently restated[2] what the procedure should be. Our water-pump is of the type recommended by him, and gives sufficient pressure to produce an air current which removes all the ammonia from 10 to 20 c.c. of N/10 NH_4Cl solution in one hour. This we have verified a number of times in the course of this work. But the aeration for the removal of the ammonia from urine has never been less than four hours, and often was kept up for a much longer time.

It will be seen that the ammonia nitrogen rose in both absolute and relative amounts on the day following labor for both cases (Table 1). In order to make certain that this was not due to contamination of the bladder by the catheter, we had the bladder thoroughly irrigated twice with a saturated solution of boric acid, warmed to body temperature, on the third day of collection by the catheter. The fact that the ammonia did not fall very appreciably the following day, proves that the bladder had been clean. The larger output in both absolute and relative amounts immediately following labor probably is due to the acid products, e. g., lactic acid,[3] formed and not completely oxidized during labor. We found no trace of acetone bodies at this time, although the total acidity was slightly higher than it had been previously.

In both cases the absolute amount of ammonia continues higher than it was before parturition. With Case 4 (Table 1) the average for the six days on which urine was analyzed previous to delivery is 0.54 gm., and for four days after delivery (neglecting the first two on account of the influence of labor), it is 0.76 gm. With Case 5 (Table 1) it is 0.61 gm. for the two full twenty-four-hour urines before delivery and 0.92 gm. for the last six days of the post-partum period (again neglecting the first two days of the puerperium). This difference between antepartum and

2. Folin: Jour. of Biol. Chem., 1912, xi, 523.

3. This sequence is known to occur in phosphorus poisoning. (Lusk: Am. Jour. Physiol., 1907, xix, 461.)

TABLE 1.—METABOLISM EXPERIMENT SHOWING COURSE OF AMMONIA EXCRETION

CASE FOUR (8). NORMAL PREGNANCY

No.	Date, 1912	Vol. c.c.	Sp. Gr.	Acidity, c.c. N/10. 10 c.c. Diluted Urine to 2,000 c.c	Albumin[1]	Wt., kg.	Max. Syst. Bl.-Pr.	Cal. per Kg. in Food	Total N	NH_3N	Per Cent. NH_3N	Remarks
	Oct.											
1	28-29	1920	1015	1.92	0	78.6	....	....	9.52	0.41	4.3	
2	29-30	1980	1020	3.2	0	.....	100	42	17.94	0.73	4.1	
3	30-31	1540	1021	3.1	0	.....	90	42	14.75	0.76	5.2	
	Nov.											
4	31- 1	660[2]	1024	2.7	0	.....	104	42	6.39	0.36	5.7	
5	2- 3	1350	.....	2.0	....	.....	110	42	11.87	0.53	4.5	
6	5- 6	1150	.....	1.3	....	.....	100	42	8.12	0.42	5.1	
7	6- 7	1100	.....	2.5	....	.....	100	36	10.92	0.73	6.5	In labor.
8	8- 9	1450	.....	3.5	....	.....	115	44	16.80	1.19	7.0	Delivered 10 a. m. All urines by cath. on Nov. 7 and 8.
9	9-10	1450	.....	2.3	....	.....	110	45	16.07	0.84	5.2	All urine by catheter.
10	10-11	1640	.....	3.1	....	.....	105	52	17.52	0.88	5.0	All urine by catheter. Bladder washed.
11	11-12	1350	.....	3.0	....	.....	107	46	12.04	0.49	4.0	Urine voided spontaneously.
12	12-13	1650	.....	3.7	....	70.1	100	47	19.88	0.84	4.2	Urine voided spontaneously.

CASE FIVE (M). NORMAL PREGNANCY

	Oct.											
1	28-29	2260	1061	1.06	+	61.6	....	....	12.15	0.61	5.0	
2	29-30	700[2]	1020	2.12	+	.....	138	43	5.84	0.29	5.4	
3	30-31	1750[2]	1015	1.78	+	.....	124	43	4.98	0.25	5.2	
	Nov.											
4	31- 1	1880	1017	2.1	++	.....	108	43	11.59	0.62	5.3	Labor Nov. 3-5. Del. Nov. 5th, 3 a. m.
5	5- 6	2000	.....	2.3	++	.....	142	40	12.86	1.28	10.0	All urine by catheter.
6	6- 7	2000	.....	1.7	++	.....	138	38	11.39	0.88	7.7	All urine by catheter.
7	7- 8	1100	.....	1.8	++	.....	128	47	17.69	1.17	6.6	All urine by catheter. Bladder washed.
8	8- 9	1850	.....	2.5	++	.....	116	48	15.98	0.98	6.1	Urine spontaneously and by catheter.
9	9-10	1650	.....	2.5	+	.....	122	49	14.89	0.79	5.3	Urine spontaneously and by catheter.
10	10-11	1400	.....	3.3	+	.....	118	50	17.08	1.05	6.1	Voided spontaneously.
11	11-12	1320	.....	2.0	+	.....	114	51	16.97	0.77	4.5	Voided spontaneously.
12	12-13	1700	.....	3.2	+	52.4	125	52	16.97	0.79	4.7	Voided spontaneously.

1. Acetone and diacetic acid absent.
2. Some urine lost.

post-partum urines in general is not due to a difference in the food. It may possibly be due to the autolysis of the uterus accompanied by the liberation of acid bodies which must be neutralized by ammonia.

The resolution of the uterus results in a gradual increase in the total output of urinary nitrogen for several days, and a greater increase on the sixth day in both cases. This confirms again the observation of Longridge,[4] which one of us[5] had already confirmed in normal cases. Because of the higher total nitrogen excretion, the percentage of the ammonia nitrogen does not increase. In fact, it is even lower for Case 4 on the days which have been averaged for the absolute amount than before delivery (4.6 per cent. as against 4.8 per cent.). In Case 5 the average for the post-partum urines, not influenced by parturition, is 5.6 per cent., and for the two full days ante-partum it is 5.2 per cent. On the whole, one may say that the percentage of ammonia nitrogen in the urine is almost the same during the puerperium as immediately before delivery.

EFFECT OF FOUL BLADDER

Contrasted with the above results are those presented in Table 2 for Cases 6 and 7. These are both normal pregnancies, no symptoms having been noted or complained of at any time previous to or immediately following our observations. Like Cases 4 and 5, they were kept on special diets, consisting of milk, eggs, bread and butter, custard and boiled rice and containing approximately 2,100 calories. The records show that the amounts taken each day were nearly the same. Case 6 had at the beginning of our observations 2 gm. of ammonia nitrogen in the urine. This fell gradually for about a week, when it had reached the level shown on the first day of the table. The striking thing about the chart, however, is the enormous fall in the ammonia nitrogen which resulted from washing the bladder on the second day following delivery. The interpretation of the rest of the chart must obviously be made in the light of this effect. Microscopical examination of the urines obtained before delivery, but made subsequent to the discovery of the contamination, revealed the presence of many bacteria and some pus cells, but it was a case which, without the confirmatory evidence of the effects of irrigation, might have been allowed to pass as an "unexplained" high ammonia.

No doubt a part of the high ammonia on the day of delivery and the day after may be ascribed, as we have just seen, to the effects of labor itself, but so great a fall—from 2.27 gm. ammonia nitrogen to 1.33 gm.—can only be attributed to the cleansing which the bladder received. It happens that the total nitrogen in the urine October 2 to 3 and October 4 to 5 is nearly the same, so that the fall to 9 per cent. in this total in the

4. Longridge: Jour. Obst. and Gynecol., 1908, xiii, 420.
5. Murlin: Surg., Gynecol. and Obst., January, 1913, p. 43.

ammonia fraction cannot be referred to a change in the diet, nor, since the autolysis is not yet far enough advanced, to a change in the uterus. That the extra ammonia came largely if not wholly from the decomposition of urea is shown by the sums of the urea and ammonia fractions for these days; and that the decomposition took place in the bladder and not in the bottle we are certain, because of the fact that the preservative

TABLE 2.—EXPERIMENTS ILLUSTRATING INFLUENCE OF FOUL BLADDER

CASE SIX (B). NORMAL PREGNANCY

No.	Date, 1912	Volume	Sp. Gr.	Acidity	Albumin[1]	Total N	Urea N	NH_3N	Per Cent. Urea N	Per Cent. NH_3N	Diet and Condition of Patient
1	Sept. 27-28	1670	.....	1.30	Tr.	12.37	9.29	1.78	75.1	14.3[2]	Special diet of milk, eggs, bread and butter, custard and boiled rice, containing about 2,100 calories.
2	28-29	1380	.....	0.80	Tr.	9.72	7.48	1.10	76.8	11.3	
3	28-29	1530	.....	2.0	....	13.00	10.04	1.35	77.2	10.3	
4	Oct. 2- 3	1860	1021	1.9	....	14.71	10.60	2.27	72.0	15.4	Baby born Oct. 2. Catheter used six hours.
6	4- 5[3]	1230	1023	0.3$_5$	0	14.08	10.56	1.33	75.0	9.4	Bladder washed.
7	5- 6	950	1020	1.75	0	12.41	9.45	1.08	76.4	8.7	

CASE SEVEN (T). NORMAL PREGNANCY

1	24-25	1800	.....	2.3	0	13.38	10.58	1.07	79.0	8.0	Third day postpartum; second day of using catheter. Special diet of milk, eggs, bread and butter, custard and boiled rice containing about 2,100 calories.
2	25-26	950	.....	1.4	0	11.39	9.20	0.82	80.7	7.2	Bladder washed.
3	26-27	1430	.....	0.8	0	13.34	10.52	0.68	78.8	5.1	

1. Acetone and diacetic acid absent.
2. In this case bacteria were found in urine before parturition.
3. The urine for October 3-4 was alkaline and is therefore not considered.

was placed in the bottle before it left the laboratory each day and because of the care which was constantly exhibited by the nurse who had charge of these cases.

Case 7 will be readily understood from the table. The fall neither in absolute nor in percentage amounts in this case is so great when the bladder was washed as it was in Case 6, but there seems to be no other explanation; for the total nitrogen in the urine was the same, and there were no unusual acid bodies in the urine which we could detect.

We have included only these two cases which showed a fall in the urinary ammonia, because they were the only ones which exhibited the same total nitrogen before and after irrigation. Several other cases were observed in which the effect of irrigation was evident. It would be foolish to assert that no clean urine can be obtained from parturient patients without washing the bladder, but it is clear from our experience that *this possible source* of contamination must now be placed alongside of others (passage of the urine over the vulva, mixture with feces, lochia, etc.), which have always been recognized. We believe the safest and surest way to avoid all dangers of contamination is the manner of collection which we have adopted, namely, drawing the urine by catheter and placing it directly into the bottle containing preservative, and washing the bladder thoroughly with boric-acid solution twice in twenty-four hours immediately following catheterization. The boric-acid wash should not be mixed with the urine, because it interferes with certain determinations. A perfectly exact termination of the twenty-four-hour period is only thus obtainable, for by suitable manipulation of the catheter the bladder can be emptied more completely than is usually possible by the voluntary efforts of a parturient woman. A first wash with sterile distilled water is advisable, in order to obtain the last traces of urine in the bladder. The procedure should be so timed that it terminates on the last moment of the twenty-four-hour period.

While this routine may seem to some unnecessary, we are certain from long experience in metabolism experiments with animals that it is the only scientific procedure in metabolism studies with bed-ridden patients.

COMPLETE NITROGEN PARTITIONS

Tables 3, 4 and 5 contain the complete analyses for the three normal cases whose metabolism was reported in summary in the previous paper. Several points of interest may be seen in the day-to-day record. For example, the influence of the changes in diet from "ordinary hospital fare" containing meat to a diet consisting largely of milk, can be seen especially in the columns for ammonia, creatin and total purin nitrogen; and the variation in all the fractions which occur normally, or the range of error, as the case may be, is exhibted.

METHODS OF ANALYSIS

Total nitrogens were determined by the Kjeldahl method, using Folin's sulphate mixture for hydrolysis; urea-plus-ammonia by Benedict's[6] method; ammonia as already stated; creatinin and creatin by Folin's colorimetric method, converting creatin to creatinin in 500 c.c. flasks provided with pipet condensers and kept on the electric stove just

6. Benedict: Jour. of Biol. Chem., 1911, viii 405.

below boiling for three hours; total purin by a modification of Hall's[7] sedimentation method, which is in principle the old Camerer-Arnstein method of precipitation with silver; and amino-acids by a modification of the "new" formalin titration method introduced by Henriques and Sörensen.[8] Only the two last-named methods require any comment.

TOTAL PURIN METHOD

We began with Hall's purinometer, but owing to the fact that the purinometer can be used for only one determination in twenty-four hours, if results at all accurate are to be obtained, we have adapted the method to the use of the centrifuge as follows: 14 c.c. of urine (the twenty-four-hour urine diluted to 2,000 c.c.), is placed in a graduated centrifuge tube and 2 c.c. of Hall's purinometer, solution No. 1, is added and the resulting precipitate centrifuged. From the supernatent fluid 11.43 c.c. (equals 10 c.c. urine) is then measured into another centrifuge tube and 2 c.c. of strong ammonia and 2 c.c. of 3 per cent. silver nitrate solution are added. This mixture should stand for one-half hour or more to allow complete reaction. A centrifuge running at 1,800 revolutions per minute will throw down all the silver-purin in about twenty minutes. This precipitate is then stirred up with a glass rod and is washed twice with a 1 per cent. solution of ammonia, being thrown down each time by running the centrifuge for about five minutes. Finally it is washed into a Kjeldahl flask, the ammonia is boiled off with magnesium oxid and the regular Kjeldahl procedure follows for direct determination of the nitrogen. Titration is best accomplished with N/50 NAOH solution.

With a six-arm centrifuge the method is fairly rapid and is certainly not less accurate than the original Camerer-Arnstein method or any of its many earlier modifications. This we have satisfied ourselves of by use of pure uric acid, xanthin and hypoxanthin.

AMINO-ACID METHOD

The amino-acid fraction was determined at first by only a slight modification of the so-called "new" method of Henriques and Sörensen. This modification consisted only in driving off the ammonia by the aeration method of Folin, running the most rapid current of air obtainable with a suction pump (nearly always over night, eighteen hours). Controls with NH_4Cl solution in equal volume of water (50 c.c.) convinced us after a time that we were not getting quite all the ammonia especially in urines containing much of it, and this conclusion led us to adopt a different method of removing the ammonia; namely, by precipitation with phosphotungstic acid.[9]

The method which we now use is as follows: Take 200 c.c. of urine and add an equal volume of 10 per cent. phosphotungstic acid in 2 per cent. HCl. Allow to sediment for at least four hours (better over night). Pour off 200 c.c. of the supernatant fluid into a 250 c.c. flask, add 1 c.c. of 1 per cent. phenolphthalein and saturated solution of barium hydrate, or barium hydrate in substance, until the solution turns decidedly pink. Make up to 250 c.c. with water, stopper, shake up thoroughly and let stand for two hours. Filter off two 100 c.c. samples (equals 40 c.c. urine each) for duplicate titrations, and follow the regular Henriques and Sörensen procedure observing all precautions.[10]

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7. Hall, I. Walker: The Purin Bodies of Foodstuffs, 1903, p. 149.
8. Henriques and Sörensen: Ztschr. f. physiol. Chemie, 1910, lxiv, 120.
9. Benedict and Murlin: Proc. Soc. for Exper. Biol. and Med., 1912, ix, 109.
10. For a good critique of this method see "Die Formoltitration," by H. Jessen-Henson in Abderhalden's Arbeitsmethoden, 1913, vi, 262.

DISTRIBUTION OF THE NITROGEN IN THE URINE OF NORMAL PREGNANCY

The data regarding the blood-pressures, temperatures, pulse and respiration in Tables 3, 4 and 5 should be convincing evidence, when taken in connection with the absence of symptoms, that these three cases were entirely normal in every respect. All of the patients were in the last month of pregnancy. They were placed in a separate ward under the care of a nurse whose main occupation was the supervision of their diets and the collection of their urines. The women did no work and were practically in a condition of rest during the course of these observations.

On the first two days and the fourteenth or last day of the observation period, they were kept on a regular ward diet which contained meat. From the third to the ninth day inclusive they received a diet of whole milk, bread and butter, and a constant amount of milk-sugar. An effort was made to keep the calories supplied proportional to the requirements of the individuals; but in this we did not wholly succeed. The requirements for women in the last few weeks of pregnancy, who are resting quietly in bed, has been found by Carpenter and Murlin,[11] by means of both the direct and the indirect methods of calorimetry, to be in the neighborhood of 30 calories per kilogram. For a fat woman it would not be more than 27 calories per kilogram.

We estimated the requirement for these patients sitting up part of the time and moving about the ward, to be not over 35 calories per kilogram. Only one (Case 2) of the three took that quantity of food for any length of time, and is the only one that shows a distinct gain in weight. But for the severe catharsis given on December 16, the patient would probably have finished the two-weeks period a couple of pounds heavier than at the start. That all of the patients were fairly well nourished, however, and that the children did not suffer, is shown by the fact that within three weeks all came to parturition and delivered children weighing as follows: Case 1, 9 pounds, 4 ounces; Case 2, 9 pounds, 8 ounces; Case 3, 7 pounds, 2 ounces.

In our preliminary paper and in a more recent contribution[5] by one of us, the general physiological significance of the different nitrogen fractions has been sufficiently considered. The questions that require further evidence and discussion are: *To what extent does pregnancy of itself alter the distribution of the nitrogen, and can such alterations be accounted for on purely physiological grounds?*

To answer the first question with any approach to accuracy it is necessary to compare the metabolism between the pregnant and the non-pregnant states either in the same individual or in different individuals

11. Carpenter and Murlin: The Energy Metabolism of Mother and Child Just Before and Just After Birth, THE ARCHIVES INT. MED., 1911, vii, 184.

in the same general health, age, etc.; and in either case on the same diet. It is desirable that the nitrogen balance should be known so that the influence of nitrogen retention may be observed. For the dog these conditions have been rigorously met by following the metabolism on the same diet throughout the entire gestation period.[12] For the woman, in addition to our studies, a paper by Landsberg[13] has appeared recently in which the author compares the metabolism as to the urea, ammonia and amino-acid nitrogens of six non-pregnant women with that of ten normally pregnant women, all kept on similar diets. Nitrogen, sulphur and phosphorus balances were kept. Heynemann[14] has done the same, omitting the balance studies, for the creatin and creatinin. Falk and Hessky[15] a short time before had made analyses for ammonia, amino-acid and polypeptid nitrogens in the urines of both pregnant and non-pregnant women, but their conditions were apparently not so carefully controlled.

We believe that we now have in these researches, together with our own, sufficient data to enable one to forecast pretty definitely for the last weeks of a normal pregnancy (for a woman whose intake of food is known and is sufficient to meet her requirements), what the distribution of the nitrogen should be.

UREA NITROGEN

It is now demonstrated[5, 12] that the retention of nitrogen in pregnancy will cause a diminution in the absolute amount of urea excreted. Both in dogs and in women it is found that where retention of nitrogen is known to be greater, or where the conditions for nitrogen retention are known to be present, the urea falls. The percentage fall is even greater than the absolute fall, because, as we shall see later, certain other nitrogenous constituents of the urine are slightly increased.

Landsberg's experiments unfortunately are largely invalidated so far as the urea determinations are concerned by the method employed; namely, taking the total nitrogen in the filtrate after removal of ammonia and precipitation with alcohol and ether (Spiro's method). Multiplying this amount of nitrogen by 15/7 is said to give the urea nitrogen. This method has long been known[16] to yield all of the creatinin and hippuric acid nitrogen, part of the uric acid and probably a large part of the creatin nitrogen (which is nearly always present in the urine of late pregnancy), in addition to the urea nitrogen. Landsberg's estimate of the fraction (82 to 86 per cent.) is therefore certainly too high, possibly as much as 6 or 7 per cent. However, even by this method, he finds the

12. Murlin: Am. Jour. Physiol., 1911, xxviii, 422.
13. Landsberg: Ztschr. f. Gebürtsh. u. Gynäk., 1912, lxxv, 163.
14. Heynemann: Ztschr. f. Geburtsh. u. Gynäk., 1912, lxxv, 110.
15. Falk and Hessky: Ztschr. f. klin. Med., 1910, lxxi, 261.
16. Howe and Hawk: Comparative Tests of Spiro's and Folin's Methods for the Determination of Ammonia and Urea. Jour. Biol. Chem., 1909, v, 477. See also Neubauer and Huppert's Analyse des Harns, 1910, p. 574.

average percentage for normal pregnancy (83.6 per cent.) lower than for normal non-pregnant women (85.02 per cent.).

The three normal cases here reported show an average of 77.8 per cent., 70.8 per cent. and 81.4 per cent., respectively. (Tables 3, 4 and 5.)[17] The lowest percentage occurs in the urine of the patient (Case 2), who received most nearly her requirement of food, who gained most in weight and was therefore probably retaining most nitrogen. The next lowest occurs in the patient (Case 1), who received the medium amount of food; and, as the figures stand, the highest percentage occurs in the case (3) that received the lowest amount of food per unit of weight. It is probable, however, that the average for Case 3 is too high (see note at bottom of table).

The same relationship holds also for the urea-plus-ammonia nitrogen, i. e., for all the nitrogen that comes from the end-products of protein digestion or from putrefaction in the alimentary canal. Such a relationship would not hold if the factor of undernutrition, which Folin[18] has shown to play an important part in determining the percentage of urea, ammonia, etc., entered here.

The urea average for the whole antepartum period, taking the three cases together, is 76.6 per cent. For the three cases reported by Murlin[5] it was 75.6 per cent. The range in all six cases is from 70.8 (Case 2 this series) to 79.4 (Case 1 former series). Taking urea and ammonia together or "total-amid nitrogen" the average for this series is 84.2 per cent.; for the former series 82.5 per cent. The range is from 80.3 (Case 2 this series) to 86.7 (Case 3 former series).

AMMONIA NITROGEN

These tables show something of the range that may be expected in the ammonia excretion from normal cases four to six weeks before delivery. Case 1 shows an average of 0.74 gm., 6.9 per cent. of the total nitrogen; Case 2, 0.54 gm., 10.3 per cent. of the total, and Case 3, 0.51 gm., or 5.3 per cent. of the total. Landsberg finds as the results of about four days' examination on ten patients that the average of all is 0.786 gm. ammonia nitrogen, or 6.8 per cent. as contrasted with 0.771 gm., or 4.3 per cent. from six non-pregnant women on a similar diet. Landsberg does not lay special stress on the fact that the absolute amount is but slightly higher and that the difference in percentage is really due to the larger output of total nitrogen (16.03 gm. in the non-pregnant subject, as compared with 12.68 gm. in the pregnant). Falk and Hessky, among the later observers, also found the relation of the ammonia nitrogen to the total nitrogen "higher than the figures which are assumed to be normal on a

17. The averages in these tables differ slightly from the averages published in our preliminary paper because three more days are here included.

18. Folin: Am. Jour. Physiol., 1905, xiii, 66.

TABLE 3.—Results of Metabolism Experiment in

No.	Date, 1911	Condition of Patient: Weight, kg.	Blood-Pr.	Temperature	Pulse	Respiration	Diet	Qualitative Examination: Cal. per kg.	Volume	Spec. Grav.	Albumin	React. N/10 NaOH
	Dec.											
1	5- 6	.....	....	97.8	108	22	Ordinary hospital fare...	.....	1050	1031	Tr.	Ac. 5 c.c.
2	6- 7	.....	115	97.8	88	22	Ordinary hospital fare...	.....	980	1032	Tr.	Ac. 5 c.c.
3	7- 8	.....	120	97.8	92	22	Milk, bread and butter..	18	768	1034	0	Ac.
4	8- 9	75.0	118	97.8	96	22	Milk, bread and butter..	24	800	1035	0	Ac.
5	9-10	75.0	116	98.0	92	22	Milk, bread and butter..	20	860	1035	0	Ac. 6 c.c.
6	10-11	75.0	110	98.0	96	24	Milk, bread and butter..	29	830	1030	0	Ac. 6 c.c.
7	11-12	74.5	96	98	96	24	Milk, bread and butter..	33	750	1033	0	Ac. 9 c.c.
8	12-13	75.4	108	98	88	24	Milk, bread and butter[2]..	37	930	1034	0	Ac. 6.8 c.c.
9	13-14	75.4	108	97.8	92	24	Milk, bread and butter[2]..	36	1085	1030	0	Ac.
10	14-15	75.0	118	98	92	24	Milk and starchy foods[2].	31	1250	1028	0	Ac.
11	15-16	74.8	118	97.8	104	22	Milk and starchy foods[2].	31	585	1026	0	Ac.
12	16-17	75.0	114	98	96	22	Milk and starchy foods[2].	31	635	1028	0	Ac.
13	17-18	75.0	110	98	102	24	Milk and starchy foods[2].	31	1010	1026	0	Ac.
14	18-19	74.8	110	98	104	24	Ordinary hospital fare...	.....	1360	1021	0	Ac.
							Average					

1. Urine bottle broken.

2. Oatmeal, bread, graham crackers, baked potato.

TABLE 4.—Results of Metabolism Experiment in

No.	Date, 1911	Condition of Patient: Weight, kg.	Blood-Pr.	Temperature	Pulse	Respiration	Diet	Urine Qualitative Examination: Cal. per kg.	Volume	Spec. Grav.	Albumin	Reaction
	Dec.											
1	5- 6	.....	....	97.6	116	24	Ordinary hospital fare.....	.....	1550	1016	Tr.	Ac.[1]
2	6- 7	.....	130	97.8	112	22	Ordinary hospital fare.....	.....	960	1016	0	Ac.
3	7- 8	.....	140	98	110	22	Milk, bread and butter.....	24	932	1020	0	Ac.
4	8- 9	65.4	115	98	112	22	Milk, bread and butter.....	28	812	1018	Tr.	Ac.
5	9-10	65.0	112	97.8	112	22	Milk, bread and butter.....	21	700	1020	Tr.	Ac.
6	10-11	65.0	106	97.8	128	32	Milk, bread and butter.....	26	710	1020	0	Ac.
7	11-12	65.0	105	98	100	24	Milk, bread and butter.....	27.5	540	1017	0	Ac.
8	12-13	65.9	112	98.2	96	24	Milk, bread and butter[4].....	38.3	740	1018	Tr.	Ac.
9	13-14	65.9	114	98	88	24	Milk, bread and butter[4].....	33.5	650	1020	Tr.	Ac.
10	14-15	65.7	116	98.2	84	22	Milk and starchy foods[2].....	35	750	1018	Tr.	Ac.
11	15-16	65.7	122	98	112	24	Milk and starchy foods.....	35	610	1026	0	Ac.
12	16-17	65.9	118	98.2	100	22	Milk and starchy foods.....	35	420[3]	1024	0	Ac.
13	17-18	65.0	106	98	96	22	Milk and starchy foods.....	35	720	1022	Tr.	Ac.
14	18-19	65.4	110	98.2	100	22	Ordinary hospital fare.....	.....	1130	1014	Tr.	Ac.
							Average					

1. Acetone and diacetic acid absent throughout.

2. Oatmeal, bread, graham crackers, baked

NORMAL PREGNANCY. CASE 1 (B. H.) NINTH MONTH

Quantitative Urine Analysis of Nitrogen Fractions

Total N, gm.	Urea N, gm.	NH_3N, gm.	Cr_1N, gm.	Cr_2N, gm.	Total Purin N gm.	Amino Acid N, gm.	Undet. N, Gm.	Urea N, Per Cent.	NH_3N, Per Cent.	Cr_1N, Per Cent.	Cr_2N, Per Cent.	Total Purin N Per Cent.	Amino Acid, N Per Cent.	Undet. N, Per Cent.
15.06	11.43	0.83	0.41	0.13	0.22	.....	.....	75.9	5.5	2.7	0.9	1.4	.....	.....
12.88	9.90	0.74	0.35	0.19	[1]	0.37	.....	76.9	5.7	2.7	1.5	.....	2.9	.....
11.54	8.91	0.78	0.39	0.15	0.41	0.40	0.42	77.2	6.8	3.4	1.3	4.2	3.5	3.6
10.86	8.73	0.68	0.37	0.14	0.31	0.43	0.20	80.4	6.3	3.4	1.3	2.8	4.0	1.8
11.37	8.76	0.71	0.38	0.06	0.25	0.34	0.88	77.0	6.2	3.3	0.5	2.2	3.0	7.8
11.28	8.70	0.71	0.34	0.07	0.28	0.35	0.92	77.1	8.1	3.0	0.6	2.4	3.1	8.1
12.07	9.27	0.88	0.40	0.08	0.28	0.50	0.66	76.8	9.4	3.3	0.7	2.3	4.1	5.4
12.43	9.48	0.88	0.36	0.09	0.36	0.45	0.81	76.3	7.1	2.9	0.7	2.9	3.6	6.5
13.33	10.54	0.88	0.38	0.05	.....	0.47	.....	79.2	6.6	2.9	0.4	.....	3.6	.....
15.57	12.99	0.78	0.41	0.16	0.64	0.53	0.04	83.4	5.0	2.6	1.3	4.1	3.4	0.2
9.02	7.21	0.57	0.27	0.19	.....	0.59	0.19	80.0	6.3	3.0	2.2	.....	6.5	2.1
8.06	6.25	0.58	0.28	0.06	0.36	0.47	0.06	77.5	7.2	3.5	0.7	3.5	5.8	1.6
10.98	8.17	0.71	0.32	0.20	0.31	0.63	0.64	74.4	6.5	2.9	1.8	2.8	5.7	2.9
8.29	6.45	0.83	0.38	0.09	0.67	0.61	0.75	77.8	10.0	4.6	1.1	8.1	7.4	9.0
11.61	9.30	0.74	0.38	0.12	0.37	0.47	0.55	77.8	6.9	3.2	1.1	3.3	4.4	4.4

3. Fifteen gr. thyroid extract daily.

NORMAL PREGNANCY. CASE 2 (M. L.) NINTH MONTH

Quantitative Urine Analysis of Nitrogen Fractions

Total N, gm.	Urea N, gm.	NH_3N, gm.	Cr_1N, gm.	Cr_2N, gm.	Total Purin N gm.	Amino Acid N, gm.	Undet. N, Gm.	Urea N, Per Cent.	NH_3N, Per Cent.	Cr_1N, Per Cent.	Cr_2N, Per Cent.	Total Purin N Per Cent.	Amino Acid, N Per Cent.	Undet. N, Per Cent.
9.01	6.52	0.76	0.42	0.0	0.75	0.29	0.27	72.3	8.4	4.7	0.0	8.3	3.2	3.0
6.49	4.90	0.51	0.27	0.05	0.43	0.15	0.18	76.5	7.9	4.2	0.7	3.0	2.4	2.8
6.49	4.72	0.65	0.35	0.06	0.34	0.28	0.09	72.7	10.0	5.4	0.9	5.2	4.3	1.4
5.45	4.02	0.51	0.28	0.11	0.25	0.26	0.03	73.7	9.3	5.1	2.0	4.6	4.8	0.4
4.65	3.28	0.47	0.24	0.0	0.22	0.37	0.07	70.6	10.1	5.2	0	4.7	7.9	1.5
5.71	4.17	0.53	0.28	0.0	0.08	0.28	0.37	72.8	9.3	4.9	0	1.4	4.9	6.4
3.42	2.38	0.36	0.14	0.0	0.22	0.18	0.14	69.7	10.5	4.1	0	6.4	5.3	4.0
5.38	3.91	0.60	0.17	0.0	0.25	0.24	0.21	72.6	11.2	3.2	0	4.6	4.4	3.9
4.81	3.35	0.59	0.16	0.13	0.20	0.20	0.18	67.6	12.2	3.3	2.7	4.2	4.2	3.7
5.10	3.55	0.54	0.22	0.11	0.28	0.35	0.05	69.6	10.6	4.3	2.2	5.5	6.9	0.9
5.82	4.13	0.57	0.21	0.0	0.31	0.20	0.41	70.9	9.8	3.6	0.0	5.3	3.4	7.0
3.36	2.11	0.58	0.23	0.0	0.17	0.26	0.11	63.8	17.3	3.9	0	5.1	7.7	3.3
5.71	3.98	0.50	0.22	0.0	0.31	0.26	0.44	69.2	8.8	3.9	0	5.4	4.6	7.7
5.43	3.70	0.44	0.23	0.0	0.20	0.35	0.51	68.2	8.1	4.2	0	3.7	6.4	8.4
5.49	3.91	0.54	0.24	0.03	0.29	0.26	0.22	70.8	10.3	4.3	0.6	4.9	5.0	3.9

potato. 3. Catharsis. 4. Fifteen gr. thyroid extra daily.

mixed diet (3 to 5 per cent.)," and their table shows many instances of a percentage higher than any that we have found except on a day of catharsis (see table).

Since the liver is the organ especially charged with the conversion of ammonia to urea, particularly that ammonia which may arise from putrefactive processes in the large intestine,[19] it has been assumed that a high ammonia, unaccounted for by any unusual acid bodies in the urine, is a sign of liver inefficiency,[20] and Falk and Hessky adopt this explanation of their findings, pointing out that the intolerance of the pregnant woman for levulose is a parallel indication.

TABLE 5.—Results of Metabolism Experiment in

No.	Date, 1911	Weight, kg.	Blood-Pr.	Temperature	Pulse	Respiration	Diet	Cal. per kg.	Urine Qualitative Examination			
									Volume	Spec. Grav.[1]	Albumin	React. N/10 NaOH 10 c.c. of Urine Diluted to 2,000 c.c.
	Dec.											
1	5-6	.....	....	98	100	22	Ordinary hospital fare.	.....	1700	1028	0[1]	28 c.c. Ac.
2	6- 7	.....	97	98	108	24	Ordinary hospital fare.	.....	1470	1020	0	3.1 c.c.
3	7- 8	.....	117	98	112	24	Milk, bread and butter.	18	722	1032	0	Ac.
4	8- 9	83.9	110	97.8	100	24	Milk, bread and butter.	20	612	1034	0	Ac.
5	9-10	83.6	115	98.2	108	22	Milk, bread and butter.	19	570	1030	0	6.4 c.c.
6	10-11	83.1	105	97.4	100	24	Milk, bread and butter.	23	1330	1020	0	3.5 c.c.
7	11-12	83.4	90	98	96	24	Milk, bread and butter[2]	27	780	1027	0	6.0 c.c.
8	12-13	84.1	96	97.8	92	24	Milk, bread and butter[2]	29	780	1026	0	6.1 c.c.
9	13-14	83.9	100	98.2	96	24	Milk, bread and butter.	30	750	1022	0	Ac.
10	14-15	83.6	106	97.8	96	24	Milk and starchy foods[4]	27.4	760	1022	0	Ac.
11	15-16	83.4	94	97.8	100	24	Milk and starchy foods	27.4	800	1028	0	Ac.
12	16-17	83.6	94	98.4	100	24	Milk and starchy foods	27.4	667	1024	0	Ac.
13	17-18	84.1	98	98.4	100	22	Milk and creatin foods	27.4	670[3]	1022	0	Ac.
14	18-19	84.1	98	98.2	96	24	Ordinary hospital fare.	.....	1140	1014	0	Ac.
												Average

1. Acetone, albumin and diacetic acid absent. 2. Error probably on urea. 3. Some urine lost.

Heynemann, however, reviews all the signs of liver inefficiency which have been accepted by different observers, including urobilinuria, faulty fat metabolism, levulosuria, etc., and reaches the conclusion that none of these have been established. He cites, for example, work tending to show that all female subjects have a lower tolerance for levulose than have male subjects with whom the pregnant have so often been compared. Landsberg seems to dispose of the high ammonia as a sign of liver ineffi-

19. Folin: Jour. Biol. Chem., 1912. xi, 161.

20. See, for example, Ewing and Wolff: Amer. Jour. Obst., 1907, lv, 289.

ciency by showing that the ammonia runs parallel to the total acidity in such urines. The excess acid he thinks may originate in the fetus.

While we are quite prepared to admit the possibility of Landsberg's explanation, it must be remembered that he has in his own figures and we have in ours only a slight discrepancy in the absolute amount of ammonia nitrogen to be accounted for. As a matter of fact, we do not regard our absolute ammonia figures as high at all. *It is only the relative or percentage amount which is high and that is partly explained at once by the lower total nitrogen excretion.* Assuming, however, that this is

NORMAL PREGNANCY. CASE 3 (E. B.) NINTH MONTH

Quantitative Urine Analysis of Nitrogen Fractions

Total N, gm.	Urea N, gm.	NH_3N, gm.	Cr_1N, gm.	Cr_2N, gm.	Total Purin N gm.	Amino Acid N, gm.	Undet. N, Gm.	Urea N, Per Cent.	NH_3N, Per Cent.	Cr_1N, Per Cent.	Cr_2N, Per Cent.	Total Purin N, Per Cent.	Amino Acid, N Per Cent.	Undet. N, or Error, Pct.
14.28	12.58	0.53	0.43	0.22	0.50	0.27	—0.25	88.1	3.7	3.0	1.6	3.5	1.9	—1.8
11.20	8.90	0.50	0.38	0.19	0.31	0.22	0.70	79.5	4.5	3.4	1.7	2.8	2.0	6.2
10.37	9.18	0.50	0.40	0.17	0.59	0.31	—0.78	88.5	4.8	3.9	1.6	5.7	3.0	—7.5[3]
10.64	8.61	0.46	0.39	0.12	0.12	0.29	0.65	80.8	4.3	3.7	1.1	1.1	2.7	6.1
8.06	6.54	0.34	0.25	0.08	0.31	0.24	0.30	81.0	4.2	3.1	0.8	3.7	3.0	3.7
11.84	9.31	0.57	0.42	0.08	0.22	0.39	0.85	78.6	4.8	3.5	0.7	1.8	3.3	2.2
10.15	8.10	0.53	0.33	0.04	0.45	0.27	0.43	79.8	5.2	3.2	0.4	4.4	2.6	4.2
10.70	9.34	0.63	0.36	0.10	0.31	0.29	—0.33	87.3	5.9	3.4	0.9	2.9	2.7	—3.1
7.95	6.44	0.45	0.26	0.08	0.59	0.24	—0.11	81.0	5.7	3.3	1.0	7.4	3.0	—1.4
10.25	8.13	0.66	0.38	0.08	0.25	0.38	0.37	79.3	6.4	3.7	0.8	2.4	3.7	3.6
11.03	8.57	0.66	0.41	0.02	0.50	0.45	0.40	77.7	6.0	3.7	0.2	4.5	4.0	3.6
8.47	6.98	0.50	0.31	0.12	0.22	0.44	—0.10	82.4	6.1	3.7	1.4	2.6	5.9	—1.2
5.88	4.56	0.36	0.20	0.18	0.20	0.29	+0.09	72.6	6.1	3.4	3.0	3.4	4.9	1.6
6.61	5.17	0.43	0.23	0.06	0.25	.....	.....	78.2	6.5	3.5	0.9	3.8	.....	.....
9.82	8.03	0.51	0.34	0.11	0.34	0.31	+0.16	81.4	5.3	3.5	1.2	3.6	3.3	+1.6

4. Oatmeal, bread, graham crackers, baked potato. 3. Fifteen gr. thyroid extract daily.

not sufficient explanation, is it not possible that the higher ammonia is due to a high relative (not high absolute) acidity? Suppose the fetus were drawing bases from the maternal blood more rapidly than acids, the effect would be to increase the ammonia in the urine of the mother, just as is known to occur when an excess of bases is excreted through the bowel.[21]

21. Cf. Steinitz: Jahrb. f. Kinderh., 1903, lvii, 689.

21a. Hoffström [Skand. Archiv. f. Physiol., 1910, xxiii, p. 326] indeed has shown that the fetus lays claim to the calcium of the food much more rapidly than to the sulphur and phosphorus.

INFLUENCE OF CATHARSIS ON AMMONIA PERCENTAGE

On December 16 two of the three patients were given calomel, salts and high colonic irrigation for the purpose of preparing the bowel for the administration of creatin the next day (see below). The tables show that the volume of urine was much reduced in Case 2, a fact which we have ascribed to the reduced absorption from the bowel. The amount of creatinin shown indicates that there was no reduction in the endogenous metabolism as the result of this treatment, but the amount of total nitrogen shows clearly that there was a very great change in the amount of protein material absorbed during the twenty-four hours. Singularly enough, the absolute quantity of ammonia on the day of catharsis was the same as the day before; but the percentage amount was enormously increased on account of the low excretion of total nitrogen. The catharsis in this patient must have been unusually severe (note the loss in weight), but there was no visible contamination of the urine from the bowel. The same phenomenon is to be seen in Case 3 on the day of catharsis, the rise in percentage being, however, very small as compared with Case 2, doubtless on account of a less complete purging. The absolute amount of ammonia was the same as the day before; and, by a coincidence, the same as for the corresponding day for Case 2. The rise in percentage for this day, while not more than might occur independently of such a cause, seems to be best explained by the fall in the total nitrogen. This emphasizes again the importance of great care in the interpretation of high ammonias.

CREATININ NITROGEN

The chemical history of this fraction of the nitrogenous waste from the body has not yet been fully revealed. It seems to be proportional in amount to the muscular development of the individual[22] and therefore to be associated in some way with the functioning of this tissue, but not with the amount of work it does.[23] It has been strenuously held by some that the creatinin of the urine bears no physiological relationship with the creatin which is always found in the watery extract of muscle and which is chemically so closely related to creatinin.[24] But the recent experiments of Towles and Voegtlin[25] on the subcutaneous administration of creatin to fasting dogs and to dogs with the Eck fistula and the observation of Myers and Fine[26] that the daily output of the dog, cat

22. Shaffer: Am. Jour. Physiol., 1908, xxiii, 1.
23. Shaffer: Idem., xxii, 445; Von Hoogenhuyze and Verploegh, Ztschr. f. physiol. Chemie, 1905, xlvi, 415; Pekelharing and Von Hoogenhuyze, Idem., 1910, lxiv 262.
24. Folin: Hammersten's Festschrift 1906, p. 1; Von Hoogenhuyze and Verploegh, Ztschr. f. physiol. Chemie, 1908, lvii, 161.
25. Towles and Voegtlin: Jour. Biol. Chem., 1912, x, 479.
26. Myers and Fine: Jour. Biol. Chem., 1913, xiv, 9.

and rabbit is roughly proportional to the percentage content of creatin in their muscles, go far toward proving a close relationship between them. Until this point is settled we shall not be able to appreciate the exact significance of creatinin; but it will be interesting at all events to see how its elimination in the pregnant woman compares with that in normal non-pregnant individuals.

Creatinin Coefficient. — The number of milligrams of creatinin nitrogen excreted in twenty-four hours, per milligram of body weight, is known, after Shaffer[22, 23] (loc. cit.) as the "creatinin coefficient." Shaffer's average value for "37 supposedly normal individuals" was 8.1, maximum 11.7, and minimum 5.4. One of Shaffer's "pathological cases" was a woman in normal pregnancy. She was of "only fair muscular development, slender and active." Her creatinin coefficient in the seventh month of her pregnancy was 6.35, early in the ninth month it was 6.75, and one week before labor was 6.2—all obtained on a creatin and creatinin-free diet. Shaffer does not state whether he regards this as a low coefficient, but does accede to the statement of Benedict and Meyers[27] based on their study of the creatinin coefficient in insane but otherwise normal women that "the creatinin coefficient of women is, in general, lower than for men." Shaffer explains this as due to the relatively large amount of fat and smaller amount of muscular tissue in the average woman and expresses the belief that "sex *per se* has no significance." So far as we are aware no other coefficients for normal or non-pregnant women have been published. Neither Van Hoogenhuyze and ten Doeschate[28] nor Heynemann,[29] who have reported extensive studies in pregnant women, give the weights of their subjects.

From the results published by one of us[5] a short time ago, it is possible to calculate the coefficient for one case who was kept on a creatin and creatinin-free food for several weeks preceding her labor. Taking the average of twenty-one days the excretion of creatinin nitrogen was 0.24 gm. The average net weight for this period was 69.2 kilograms, which gives a coefficient of 3.5 — but little higher than that found by Benedict and Myers for a woman convalescent from typhoid (namely, 3.1).

The average coefficients for that portion of the period of observation when the subjects of the present series were on a creatin and creatinin-free food (eleven days) are: 4.7 for Case 1, 3.2 for Case 2 and 4.7 for Case 3. It is probable that all four of these cases carried more body fat

27. Benedict and Myers: Am. Jour. Physiol., 1907, xviii, 377.

28. Von Hoogenhuyze and ten Doeschate: Ann. de Gynéc., 1911, Series 2, VIII, 17 and 97.

29. Heynemann: Ztschr. f. Gebürtsh. u. Gynäk., 1912, lxxi, 110.

than Schaffer's "slender women," which no doubt explains in part the discrepancy between his coefficient and ours. Even his case, however, shows a lower coefficient than the average for a normal man, and, if sex *per se* plays no part, lower also than for the non-pregnant woman.

One reason for this will be apparent at once; namely, the dilution, if we may call it such, of the body weight by the uterine contents. Myers and Fine[28] have recently confirmed for the cat the observation originally made by Dörner[30] for rabbits, and later by Mellanby[31] for both rabbits and chickens, that young individuals have less creatin in their muscles than adults of the same species; and Rose[32] has found the creatin content of the muscles of the new-born infant to be only 0.07 per cent., as against 0.3 per cent. for the adult. Mendel and Leavenworth[33] have furnished us the only determinations yet made for the embryo, in the case of the pig, reporting .03 per cent. for an embryo of 265 mm. length as compared with 0.45 per cent., reported by Van Hoogenhuyze and Verploegh[34] for the muscles of the grown animal. Presumably the same relationship would hold for the human embryo of corresponding age, and the creatin content would gradually increase up to 0.07 per cent. found for the new-born infant.

We have been unable to find any analyses of the pregnant uterus for creatin. Judging by the analysis of the non-striated muscle of the stomach, bladder,[35] retractor muscle of the penis[36] and for the intermediate type of muscle in the heart,[37] it should be considerably less than that of striated muscle.

If creatin is the mother substance of creatinin in the urine, therefore, neither the fetus nor the uterus may be supposed to contribute much of it. The placenta, presumably, and the membranes and the fluids certainly, would contribute none at all. All of this weight would serve, as does the body fat, to reduce the creatinin coefficient. Even if creatinin does not come from creatin, we know from the fact that the elimination of creatinin by the new-born child is very small, that the production of this substance by the fetus must be negligible.

Support for this view is found also in the following observations, some of them unpublished, on a pregnant dog.[12]

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30. Dorner: Ztschr. f. physiol. Chem., 1907, lii, 264.
31. Mellanby: Jour. Physiol., 1908, xxxvi, 447.
32. Rose: Jour. Biol. Chem., 1911, x, 265.
33. Mendel and Leavenworth: Am. Jour. Physiol., 1908, xxi, 100.
34. Von Hoogenhuyze and Verploegh: Ztschr. f. physiol. Chemie, 1905, xlvi, 432.
35. Saiki: Jour. Biol. Chem., 1908, iv, 483.
36. Buglia and Constantino: Ztschr. f. physiol. Chemie, 1912, lxxxi, 122.
37. Fürth, V., and Schwarz: Biochem. Ztschr., 1911, xxx, 413.

ON CREATIN AND CREATININ FREE FOOD

Week of Gestation.	Weight, kg.	Creatinin per Twenty-Four Hours, mg.	mg. per kg.
III	13.7	118	8.6
VII	15.2	118	7.7

ON DIET CONTAINING 275 GM. BEEFHEART

Week of Gestation.	Weight, kg.	Creatinin per Twenty-Four Hours, mg.	mg. per kg.
IV	13.9	135	9.7
IV	16.5	135	8.2
Third day after parturition	13.9	147	10.6

The return of the weight to 13.9 kg. so soon after delivery of the pups shows that the increase in weight in pregnancy was due mainly, if not entirely, to the product of conception and not to body fat. The rise of the coefficient after parturition is due to the discharge of some creatin from the involuting uterus in the form of creatinin.

This explanation may not be wholly adequate for the human organism. It will be impossible to determine whether it is or not until a rigid comparison is made between pregnant and non-pregnant women, whose weights are known, on proper diets.

Our observations on these cases having terminated nearly three weeks previous to delivery, the rise in creatinin excretion which was previously reported[5] as occurring in the last week or ten days of pregnancy does not appear here.

The creatinin and creatin metabolism just before and just after parturition has been fully discussed in former publications. Heynemann's work within the year has confirmed the conclusions we had already reached as to most of the points in discussion, and particularly as to the bearing which any alterations in this metabolism in normal pregnancy might have on the question of liver inefficiency. He argues, as did Murlin, that if it were shown satisfactorily that the liver does produce creatinin from creatin, and if it were shown further that in pregnancy the increase in creatin output is accompanied by a decrease in creatinin output, we might feel justified in accepting this as a sign of liver inefficiency, as do Van Hoogenhuyze and ten Doeschate. The former proposition, however, seems to be growing less rather than more secure as evidence accumulates, and on the latter there is no evidence at all as yet; for the tables of Van Hoogenhuyze and ten Doeschate do not show a decrease in the creatinin any more than do those of Heynemann or Murlin, and if they had followed the excretion of creatinin on a constant diet up to the day of parturition it is probable that they also would have seen a rise in this constituent rather than a fall. Heynemann's figures for the creatinin output vary so much from day to day that it is impossible to say whether his results confirm or refute his argument.

We are not inclined to lay much stress on the results from Eck fistula, especially if the hepatic artery be not tied; for Fischler[38] has shown that neither the formation of bile nor the carbohydrate tolerance is affected by this operation. Nevertheless, the newer experiments of Towles and Voegtlin[25] seem to show quite conclusively that whatever damage is done to the metabolic processes by this operation the transformation of creatin to creatinin is not one which suffers. They conclude that the liver is not an organ of prime importance in connection with the metabolism of these substances.

CREATIN NITROGEN

There is general agreement among the observations of Van Hoogenhuyze and ten Doeschate, Heynemann and ourselves as to the appearance of creatin in the urine of late pregnancy, even on a creatin and creatinin-free food. The three cases of the present series fall into line with previous ones. Case 2, however, shows less creatin than the others, and we can find no other explanation than the fact that the patient took more food per kilogram of body weight than the others. The appearance of creatin on the ninth and tenth days of the period may be the result of thyroid feeding on the eighth and ninth days. All the patients received on these days 15 grains of dried thyroid extract. This was the only case which showed any effect, and the only effect which any case showed.

It is possible that by feeding a larger amount of carbohydrate the urine could have been kept clear of creatin throughout. Mendel and Rose[39] have shown that for the rabbit at least a small amount of carbohydrate is sufficient to prevent or to diminish greatly the excretion of this constituent in the urine of otherwise fasting animals. As suggested in a former paper,[5] it is possible that the appearance of creatin in the urine of late pregnancy is due to the rapid diffusion of dextrose, the carbohydrate of the blood, through the placenta and its fixation or combustion there, thus depriving the maternal organism of its protecting influence. On December 16, the twelfth day of the experiment period and the day following thorough cleansing of the bowel, we fed two of the three patients (Cases 2 and 3) each 1 gm. of pure creatin,[40] the object being to test the capacity of the pregnant organism to eliminate or to utilize this form of nitrogen. Out of the 320 mg. of nitrogen administered, we recovered only about 60 mg. from Case 3, and none at all from Case 2 (a small quantity of urine was lost, however, from Case 3). Our hope was that by thoroughly cleansing the bowel and thereby reducing the

38. Fischler, cited by Wolf: Jour. of Biol. Chem., 1912, x, 473.

39. Mendel and Rose: Jour. Biol. Chem., 1911, x, 213.

40. The creatin was prepared for us by Dr. Stanley R. Benedict who also suggested the catharsis as a preparation for its administration.

possibility of bacterial decomposition a larger amount of the creatin fed might pass into the urine. The experiment is not very conclusive, but is in accordance with the previous results of Folin and Van Hoogenhuyze and Verploegh to the effect that creatin in the food is largely destroyed or is retained in the body of normal persons.

TOTAL PURIN NITROGEN

The total purin nitrogen excreted includes not only the nitrogen of uric acid, but also that of the less completely oxidized purin bases, hypoxanthin, xanthin, adenin and guanin, and that of any methylated compounds of this series like caffein, theobromin, etc.

Excluding coffee, tea, etc., which might contribute methylated compounds to the urine, the amount of total purin nitrogen excreted on a purin-free diet is the best measure we have of nuclein metabolism in the body, i. e., of the destruction of cell nuclei. Following the terminology of Burian and Schur,[41] this is known as endogenous nuclein metabolism.

There is still a difference of opinion whether the uric acid should be regarded as an end stage in the metabolism of nucleins[42] or as only an intermediary stage on the way, by uricolysis and oxidation, to urea.[43] Concerning the other purins there is no doubt that they could be oxidized to uric acid if opportunity offered, so that the amount in which they appear in the urine is more or less fortuitous.

The amount of purin nitrogen excreted by the pregnant subject is a matter of some interest because of the important part which nuclei play in embryogenesis and histogenesis.

Our normal subjects were kept for eleven days on a diet which was nearly, if not quite, purin-free. Tea, coffee and cocoa were excluded. The output of total purin nitrogen, then, for these eleven days is a measure—a rough one probably—of the extent to which cells are being broken down and their nucleins destroyed. The tables give the average elimination for the entire fourteen days. When meat was ingested on the first two and last days, considerably more purin was found in the urine. Taking the average of the purin-free days we find that Case 1 eliminated on the average 0.36 gm., Case 2, 0.24 gm. and Case 3, 0.31 gm. purin nitrogen; or, expressed as a coefficient in milligrams per kilogram of body weight, Case 1, 4.8; Case 2, 3.7, and Case 3, 3.7.

The only experiments which we have been able to find on purin-free diets in which the total purin nitrogen was determined by a reliable method, are those of Cathcart[44] (Camerer-Arnstein method) on the

41. Burian and Schur: Pflüger's Arch. f. path. Anat., 1901, lxxxvii, 239.
42. Wiechowski: Arch. f. exper. Path., 1909, lx, 185.
43. Cf. e.g. Frank and Schittenhelm: Ztschr. f. physiol. Chemie, 1909, lxiii, 269.
44. Cathcart: Biochem. Ztschr., 1907, vi, 109.

professional faster Beauté, and those of Mendel and Lyman[45] (method of Krüger and Schmid) on normal male subjects.

Beauté, on an egg and milk diet, excreted 0.194 gm. of total purin nitrogen just before a fast of fourteen days, and 0.166 gm. on the same diet just after the fast. In milligrams per kilogram of body weight the coefficient was 3.0 and 2.8, respectively.

In Mendel and Lyman's experiments their subject, W. H. H., excreted 0.139 gm. total purin nitrogen on a purin-free diet, and J. F. L. excreted 0.144 gm. The coefficients per kilogram were 2.4 and 2.1, respectively.

It appears that the pregnant woman excretes not a little more purin nitrogen per unit of weight than does the normal man. It has long been known that the new-born infant excretes much more uric acid per unit of weight than does the adult, and more also in the relation to the urea excretion. Both Flensburg and Rensing[46] find the explanation of this fact in the hyperleukocytosis and the consequent high destruction of leukocytes going on in the infant's body during the early days of extra-uterine life. This hyperleukocytosis occurs also in the fetus and it is known that the leukocytes are destroyed both in the spleen and in the connective tissues.[47] Hence we should expect a rapid production of both uric acid and of purins which must be eliminated through the maternal system, since Mendel and Leavenworth[48] found no uricolytic enzyme in the embryo pig.

AMINO-ACID NITROGEN

Following the caution of Henriques and Sörensen, we wish to emphasize the fact that the fraction of nitrogen actually determined by the titration method includes some nitrogen in the form of polypeptids or other combinations; nevertheless it is customary to include all such free-acid groupings as can be set free for titration by neutral formaldehyd solution, after removal of ammonia, under this designation, and the method of Henriques and Sörensen certainly gives us as close an approximation to the free amino-acids as any that we have at present.[49]

Recent determinations of the amino-acid nitrogen in pregnancy by Falk and Hessky[15] and by Landsberg[13] working by the so-called "new method," show that this fraction is slightly increased over the amount found in the urine of the non-pregnant woman on a similar diet. The former observers found the average from non-pregnant subjects to be 0.17 to 0.29 gm. in twenty-four hours, or 1.9 to 2.8 per cent. of the total

45. Mendel and Lyman: Jour. Biol. Chem., 1910, viii, 115.
46. Quoted by Czerny and Keller: Ernährung des Kindes, 1906, i, 216.
47. Minot: In Keibel and Mall's Embryology, 1911, ii, 504.
48. Mendel and Leavenworth: Am. Jour. Physiol., 1907, xx, 97.
49. A comparison of the gasometric method of Van Slyke with the titration method of Henriques and Sörensen has been made on a variety of urines by Levene and Van Slyke. Jour. Biol. Chem., 1912, xii, 309.

nitrogen. Landsberg reports 0.4 to 0.48 gm., or 2.5 to 3.0 per cent., of the total nitrogen. These figures agree fairly well with the percentage given by Henriques (2.2 per cent.) and with that by Levene and by Van Slyke[49] for normal urine.

In normal pregnancy Falk and Hessky find from 2 to 8 per cent. of the total nitrogen may be present in the form of amino-acids and Landsberg makes the figures from 0.23 to 0.67 gm., or 2.4 to 4.9 per cent. of the total. It will be observed that the absolute amount ranges even lower in the pregnancy urine than in that from non-pregnant women. Landsberg himself is disinclined to lay much stress on the very small increase which some cases show, and correctly refers a portion of the relative increase to the lower total nitrogen.

It will be seen from Tables 3, 4 and 5 that our results also show but a small increase over the amount considered normal. With Case 1 we have as the average of thirteen days, 0.47 gm. amino-acid nitrogen, or 4.4 per cent. of the total; with Case 2, for fourteen days, 0.26, or 5 per cent. of the total; and with Case 3 for thirteen days, 0.31 gm., or 3.3 per cent. of the total. All of these come within the range given by Landsberg. We call special attention to the fact that the lowest average absolute amount (0.26 gm.) is at the same time, owing to the low output of total nitrogen, the highest relative or percentage amount (5 per cent.). This shows how much the nitrogen retention may have to do with the percentage.

The agreement among all observers using a reliable method therefore is very close, and the lower figures should supercede those of Van Leersum,[50] who used his own modification of the old Schöndorff-Pfaundler method, and those of other observers who have denominated the rest nitrogen, after determination of urea, ammonia, creatinin and uric aid, as the "amino-acid nitrogen."

Falk and Hessky consider that their figures show a sufficient increase to indicate a distinct disturbance of metabolism and place their results on the side of those who regard the liver as the seat of this functional disturbance. In refutation of this view it is sufficient at this time to cite the new work of Folin[51] and his pupils and that of Van Slyke and Meyer,[52] which appear to demonstrate conclusively that neither the intestine nor the liver has any more to do with deamination of absorbed proteins than have other tissues, if they have as much. *High amino-acid nitrogen, therefore, can no longer be cited as proof of defective deamination in the liver.*

50. Van Leersum: Biochem. Ztschr., 1908, xi, 121.

51. Folin and Denis: Jour. Biol. Chem., 1912, xii, 87 and 141.

52. Van Slyke and Meyer: Jour. Biol. Chem., 1912, xii, 399.

It is only where an absolute increase of one- or two-tenths of a gram of amino-acid nitrogen or a relative increase of more than 2 per cent. can be shown, that any explanation, other than that of individual variation, analytical error or redistribution caused by retention of urea nitrogen, is called for. While we have no non-pregnancy cases for direct comparison, we doubt very much whether our results show any further departure than this. If, however, we accept the slightly higher results of Falk and Hessky we believe that we are bound to search for the explanation in some physiological peculiarity of pregnancy before we declare the result a pathological one.

In attempting to account for the condition of minus nitrogen balance which occurs in the dog at about the time corresponding to the period of morning sickness in the woman, one of us,[53] three years ago, offered the hypothesis that this period "simply marks the culmination of the more or less indiscriminate action of enzymes produced by the fetus" (fetal placenta) before the maternal placenta had been elaborated for the purpose of limiting the enzymes. Bar[54] had suggested a similar explanation (*une veritable mobilization des albumins*) for the loss of nitrogen, but did not commit himself as to the part played by the placenta.

Everything that we know about the implantation of the ovum certifies to its capacity to digest by proteolytic enzymes the uterine mucosa, and the morphological pictures which are presented by the process of vilus formation[55] bear witness that the trophoblastic cells which become the syncytial layer on the villus retain this capacity for a considerable time.[56] The demonstration by Graefenburg[57] of such enzymes in the human placenta, especially in the early months of pregnancy, and the recent discovery of Abderhalden[58] of enzymes in the blood of the pregnant woman, dog, cow and other animals, capable of splitting placental proteins, constitute significant support for the general view. Williams and Pearce[59] have found that these enzymes occurring in the blood of the pregnant woman are not specific, but are capable of acting on other proteins as well, and it is our belief that their presence in the blood is, as Abderhalden[60] admits to be possible, "simply a consequence of the commerce going on between mother and fetus, and signify only that a

53. Murlin: Am. Jour. Physiol., 1910, xxvii, 177.
54. Bar: Leçons en pathologie obstetrical, Paris, 1907, ii, 288.
55. Bruce and Teacher: Contributions to the Study of the Early Development and Imbedding of the Human Ovum; Glasgow, 1908.
56. Marshall: Physiology of Reproduction, New York, 1910, p. 484.
57. Graefenburg: Ztschr. f. Gebürtsh. u. Gynäk., 1910, lxv, 1.
58. Abderhalden and Kiutsi: Ztschr. f. physiol. Chemie, 1912, lxxxvii, 4.
59. Williams, P. F., and Pearce, R. M.: Proc. Soc. f. Exper. Biol. and Med., 1913, x, 73.
60. Abderhalden: Ztschr. f. physiol. Chem., 1912, lxxxi, 96.

very extensive transformation must take place before the material of the maternal blood can be carried over to the fetal blood." The action of placental enzymes has been offered by one of us also as an explanation of the higher amino-acid content of the urine of pregnancy.[61] If products of proteolysis going on in the placenta were diffusible into the maternal blood some of these products would pass to the kidney before there was an opportunity of their being deaminated or stored in the maternal tissues. The amount passing out by the kidney would not depend in any special way on liver function, but on the quantitative relation between their escape from the placenta and their destruction or fixation in the maternal tissues.

UNDETERMINED NITROGEN

Adding together all these fractions — urea, ammonia, creatin, creatinin, total purin and mono-amino-acid nitrogens — there yet remains a small fraction undetermined. Of course, we must recognize the fact frankly that what remains may be error. If an error of only 0.5 per cent. should occur in each of the determinations made and all of the errors were by chance in the same direction, there might be an apparent undetermined nitrogen of over 3 per cent. Assuming, however, that in most instances such errors would counterbalance each other, we find left in almost all of the urines we have examined an undetermined fraction of from 3 to 9 per cent. A very small part is in some cases due to albumin; in a few urines we have determined what Henriques and Sörensen call the polypeptid nitrogen; i. e., extra amino-acid nitrogen after digestion of the urine with strong hydrochloric aid. The indications are that this would account for a considerable part of what we have called in our tables the "undetermined nitrogen." Henriques and Sörensen have found such a fraction in normal urines. Falk and Hessky[15] have determined the polypeptid nitrogen in the urine of both pregnant and non-pregnant women and have found an increase of from two- to three-fold the normal amount in the former. Their percentage figures show about the same range as our percentage figures for the undetermined nitrogen. They believe the increase is due to an extra production of glycocoll in combination with aromatic and hydro-aromatic compounds similar to hippuric acid. We have no data from non-pregnant subjects with which to compare our results for this fraction. Granting, however, that it is increased, it seems to us more probable that compounds of the nature of oxyproteic acid, which Salomon and Saxe[62] have demonstrated to be present in the urines of normal preg-

61. Murlin: Am. Jour. Physiol., 1911, xxviii, 450.
62. Salomon and Saxe: Cited by Falk and Hessky. See note 15.

nancy in larger quantity, or the non-dialysable substances which Savaré[63] found to be increased in such urines, are responsible.

The fact that the polypeptid nitrogen falls within twenty-four hours after delivery, as shown by Falk and Hessky, we should interpret to signify that it has its source in some function or process which ceases ordinarily with complete delivery of the child and placenta. This fact alone we should think would be sufficient to absolve the liver from responsibility quite aside from the new evidence that this organ has no special function to perform in relation to amino-acids or polypeptids; for it is difficult to see how it could recover its function completely in twenty-four hours. Still less could it be supposed to recover within this time if autolytic processes or degeneration of any other type within the liver parenchyma were the determining cause. Falk and Hessky were unable to find any polypeptid nitrogen in the press-juice of a fresh placenta and concluded from this that the placenta could not be the source of such bodies in the urine. Forty per cent. of the total nitrogen in this press-juice was in the form of amino-acid. This is an important fact in connection with the source of amino-acid nitrogen of the urine. Suppose, however, the amino-acids were to escape from the placenta together with proteolytic enzymes, some of which have been proved to be reversible in their action, i. e., can build up polypeptid compounds as well as split them into their component amino-acids. Might not synthetic compounds be formed either in the circulation or in the kidney which would pass through into the urine? The sudden decline of this fraction after parturition, at all events, would seem to be better explained by a fetal (placental) origin than by a maternal origin.

SUMMARY AND CONCLUSIONS

1. In a perfectly normal pregnancy and puerperium the percentage of ammonia nitrogen in the urine before and after labor shows but slight differences and lies within normal limits (4 to 6 per cent.), except for one or two days immediately following delivery, when it is slightly increased (7 to 10 per cent.).

2. Further evidence is presented that high ammonias may be encountered in normal cases due to contamination of the bladder. Irrigation of the bladder twice in twenty-four hours with saturated solution of boric acid caused reductions from 2.27 gm. to 1.33 gm. and from 1.07 gm. to 0.68 gm. ammonia nitrogen in twenty-four hours, the food and total nitrogen being the same.

3. Complete nitrogen partitions for three normal cases over a period of fourteen days in the ninth month of pregnancy are given. From these

63. Savare: Hoffmeister's Beitr. z. chem. Phys. u. Path., 1907, ix, 401.

results and from those of other cases previously reported, we deduce the following:

4. The percentage of total nitrogen eliminated as *urea* by a pregnant woman in the last month is somewhat lower than from a non-pregnant person, due largely to the retention of nitrogen for growth of the product of conception. The average for our three cases for fourteen days is 76.6 per cent. The range for six cases is from 70.8 per cent. to 79.4 per cent.

5. The *ammonia nitrogen* in perfectly normal pregnancies, in which the retention of nitrogen is large and the total nitrogen in the urine consequently small, may range as high as 12 per cent. of the total and be unaccompanied by any unfavorable symptoms. Immediately after severe catharsis it may reach the height of 17 per cent., because of the diminished absorption of nitrogenous food from the alimentary tract, and consequently diminished excretion of total nitrogen. For this reason and because of the variations in the elimination of total nitrogen due to variations in the diet, it is inadvisable to rely on percentage figures in the determination of the ammonia, a point long ago insisted on by F. Müller. The absolute values are much more reliable. In our experiments the absolute amount varies but slightly from day to day, and previous to delivery never exceeded 0.88 gm. in urine known to be uncontaminated. From these and earlier studies we are disposed to look on any amount up to .012 gm. ammonia nitrogen per kilogram of body weight in the twenty-four-hour urine as well within normal limits.

6. The *creatinin coefficient* of normal pregnancy is much lower than the creatinin coefficient of normal male subjects and is probably lower than that of normal non-pregnant women. This is due in part to the "dilution" of the body mass in pregnancy with material (fetus, fluids, membranes, uterine muscle,[64] etc.) which contain little or no creatin.

7. The occurrence of *creatin* in the urine of normal pregnancy is confirmed in the present cases. Its appearance, however, seems to be dependent, to some extent at least, on the amount of food taken.

8. The *total purin nitrogen* is slightly higher in the urines of normal pregnancy than in the urines of normal male subjects on similar diets. An explanation is found in the same facts which account for the high elimination of uric acid by the new-born infant.

9. The *amino-acid nitrogen*, as determined by the improved formol titration method, is but slightly increased above the amount considered normal in the urines of non-pregnant subjects. This slight increase may

64. According to E. Mellanby [Proc. Roy. Soc. (B) 1913, lxxxvi, 88], the uterine muscle contains no creatin at all, and that it cannot be the source of the increased output of creatin in urine postpartum Mellanby believes he has demonstrated by collection of urines after hysterectomy.

be accounted for by supposing that proteolytic products, derived either from the placenta or from maternal tissues by action of placental enzymes, are carried by the circulation in larger amounts than usual to the kidney which is permeable to them.

10. The *undetermined nitrogen* is probably to be accounted for by polypeptid bodies, including oxyproteic acids.

11. In general, we believe that peculiarities in the composition of the urine of normal pregnancy as regards its nitrogenous constituents may be accounted for on purely physiological grounds.

THE RENAL COMPLICATIONS OF HEMATIN INTOXICATION AND THEIR RELATION TO MALARIA *

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CHAPEL HILL, N. C.

Descriptions of the paroxysm[1] and of the changes in the blood[2] produced by the intravenous injection of alkaline hematin and their relation to human malaria have been presented in previous articles, and it is the object of this paper to add a third group of conditions which may properly be described as the renal complications of hematin intoxication.

The description of these renal complications is based on a study of two classes of material: First, the urine and kidneys of a large series of rabbits, in which the production of renal lesions and disturbances of function were not the objects of the experiments, but were merely incidental; and second, twenty rabbits, in which the production of renal complications was made the prime object of the experiments.

The hematin and hematin solutions used in these experiments were the same as in former experiments. The animals were fed, for the most part, on a variety of green foods, with hay and ground grains, and allowed to take water at liberty. The food in a given experiment was kept constant as to quality throughout the experiment, and the amount of water consumed during twenty-four hours was determined by supplying measured amounts in vessels so arranged that none could be wasted. The water remaining in the vessel at the end of twenty-four hours was measured and corrections for evaporation were made from control vessels. In only a few experiments were definite amounts of water given by stomach tube. The urine collected from metabolism cages was used for routine study, but the qualitative findings were confirmed by bladder urine in many instances. Observations were made on the normal urine for one or two days before the injection of hematin. In this connection it should be noted that the daily amount and character of rabbit's urine is so variable that but little importance can be attached to slight variations in the amount of urine, the urine-water ratio or the character of the urine. The results of the hematin experiments were controlled by a

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* From the Pathological Laboratory of the University of North Carolina.
1. Brown, Wade H.: Jour. Exper. Med., 1912, xv, 579.
2. Brown, Wade H.: Jour. Exper. Med., 1913, xviii.

series of animals that received an equivalent volume of alkaline salt solution under the same conditions as those receiving hematin.

The extreme irregularity in the results of these experiments has shown conclusively that no given dose or combination of doses of hematin can be expected to p.oduce a given effect on the kidneys of the rabbit, and the results reported are to be interpreted as the most probable effect from a given degree of hematin intoxication.

THE URINE

Amount.—A single dose of 10 mg. of hematin per kilo of body weight, or less, will produce no alteration in the daily amount of urine. If such doses are repeated from day to day, however, slight increases in the output of urine and in the ratio of the urine to the water consumed are gradually developed. The increased volume of urine is also present where slightly larger doses of hematin are administered, but as the dose approaches 20 mg. per kilo, there is a distinct decrease in both the daily amount of urine and the ratio of the urine to the water consumed. In exceptional instances there may be a complete suppression of urine for twenty-four hours or longer. On discontinuing the hematin, this phase of decreased output of urine gives place to increased urinary secretion and to an increase in the urine-water ratio, both of which exceed the normal within two to three days. This change is more enduring — in one animal it persisted for twenty-one days, when observations were discontinued.

Color.—The urine in hematin intoxication is highly colored, varying from a brownish-yellow to a dark reddish-brown and occasionally shows a greenish fluorescence. A smoky-red urine is seen in some instances.

Acidity and Specific Gravity.—The reaction and the specific gravity of the urine show no constant alterations. Both of these features vary widely in the rabbit under normal circumstances, and the variations after injections of hematin are therefore difficult to relate to any action of hematin.

Albumin and Casts. — A trace of albumin and a few hyalin or granular casts may appear in the urine after even a single injection of 10 mg. of hematin per kilo of body weight. The daily repetition of this dose of hematin seldom fails to produce an albuminuria with casts. As much as 20 mg. of hematin per kilo of body weight will produce a marked albuminuria with an abundance of hyalin, granular and epithelial casts, or even blood casts. Neither the amount of albumin nor the number or variety of casts, however, can be predicted from the amount of hematin injected.

Blood and Hemoglobin.—The most interesting phase of disturbed renal function, referable to hematin poisoning, is the occurrence of

hematuria and hemoglobinuria. Severe intoxication with hematin frequently results in hematuria of a variable degree. The amount of blood may be so slight as not to be suspected from the appearance of the urine, or so great as to give the urine a distinct red or smoky color. The condition is occasionally produced by a single large dose of hematin (20 to 25 mg. per kilo.); it occurs with greater frequency from the maintenance of a given concentration of hematin in the circulation for twelve to twenty-four hours. This can be done best by injecting three or four doses of 10 to 15 mg. of hematin per kilo within such a period of time. The test on the vitality of the animal is a severe one, and the size of the dose and frequency of repetition must be judged by the condition of the animal; even then many fatalities will result before the desired effect is produced.

Free hemoglobin in the urine, not associated with hematuria, has been observed in a very few instances. Hemoglobin was demonstrated by the guaiacum test in voided urine and bladder urine in five animals out of fifty examined. In two of these cases the guaiacum test was confirmed by the spectroscope. Three other animals gave a positive guaiacum test from voided urine which was not confirmed by bladder urine. Of these eight animals, all except three showed, post mortem, either hemorrhagic lesions of the kidneys, extensive hemorrhage into the peritoneal cavity, or both, which might have accounted for the presence of hemoglobin in the urine. Of the three undoubted instances of pure hemoglobinuria, one occurred in the course of a chronic intoxication, and the other two from the repeated injection of 20 to 25 mg. of hematin twice daily; one animal developed hemoglobinuria after the fourth injection, and the other after the seventh. All efforts to devise a method of administering the hematin that would produce this condition in a greater percentage of cases proved futile. Most of the attempts resulted in the production of hematuria. It is evident, therefore, that there are other factors than the hemoglobinuria that are essential to the production of a high concentration of hematin in the circulation.

THE KIDNEYS

Mild hematin intoxication, whether acute or chronic, produces no characteristic lesions in the kidneys. There is a slight increase in the size of the organs and a slight brown pigmentation. The epithelium of the convoluted tubules and of the ascending loops of Henle usually shows parenchymatous degeneration. In the chronic cases a few hyalin casts in the tubules and foci of round-cell infiltration in the boundary zone and in the cortex also furnish evidence of injury.

The injection of large doses of hematin, however, may produce very profound and characteristic lesions. In the acute stages the kidneys

are much enlarged and are uniformly dark-brown or purplish-red in color, or are diffusely mottled with small opaque yellow areas of necrosis and areas of hemorrhage. The kidneys are moist, the cortex is thickened and the straight vessels and glomeruli are intensely congested. The boundary zone usually shows the most pronounced congestion and irregular streaks of hemorrhage may extend from this zone into both the cortex and medulla. In rare instances, small infarcts are present. The most characteristic alterations are found in the glomeruli. The glomeruli are enlarged and their vessels are enormously dilated and congested. In some instances, many of the glomerular capillaries are occluded by hyalin masses that stain a brassy-red with eosin; such glomeruli are apt to show hemorrhage into the capsular space. On the other hand, the glomerular tuft may completely obliterate the capsular space. The glomerular epithelium is very slightly swollen and a few desquamated cells are present. To a less degree, other vessels of the kidney are dilated and congested, while many of the smaller vessels show hyalin thrombi or emboli. Greenish-brown pigment is found, especially in the glomerular capillaries, both as free granules and masses and within phagocytic cells.

The uriniferous tubules, more particularly the convoluted tubules and the ascending loops of Henle, show marked parenchymatous degeneration with desquamation of the epithelial cells, or even foci of necrosis. In extreme instances almost the entire cortical system may be necrotic. Occasionally, the tubular epithelium will show granules of greenish-brown pigment, much of which, as it reacts for iron only after oxidation with hydrogen peroxid[3] and is soluble in dilute alkalies, must be regarded as hematin. This pigmentation is more common in living than in necrotic cells. An abundant albuminous precipitate and many varieties of casts are found in the tubules throughout the kidney. Hemorrhage into the tubules is found in many cases of extreme intoxication. The hemorrhage is usually patchy in its distribution — seldom diffuse — and occurs in the region of those glomeruli that show hemorrhage or occlusion of their vessels. In some of these cases, there is also a diffuse staining of the epithelium with hemoglobin and granules or droplets of hemoglobin are found in the cells, in the lumen of the tubules and in the capsular space. The presence of hemoglobin was observed in three instances in which there was no hemorrhage. The presence of hemoglobin was verified by oxidation with hydrogen peroxid and obtaining an iron reaction, and by the presence of brownish, granular and crystalline deposits in the cells and lumen of the tubules in specimens fixed in a solution of formaldehyd immediately after the death of the animal.[4]

3. Brown, Wade H.: Jour. Exper. Med., 1911, xiii, 477.
4. Browicz: Virchows Arch. f. path. Anat., 1900, clxii, 373.

The interstitial tissues are usually edematous and there may be foci of hemorrhage. Foci of necrosis are usually surrounded and invaded by polymorphonuclear leukocytes.

In two instances anemic infarcts were observed from occlusion of relatively large vessels by masses of hyalin material.

In the early stages of recovery from hematin intoxication and at the time the kidneys are showing an increased urinary secretion, several important changes from the above description are to be noted. The glomerular tufts now decrease to normal size or are shrunken, while the capsular spaces and tubules are dilated and contain desquamated epithelial cells and an abundant albuminous precipitate.

In chronic intoxications, and as recovery from acute intoxications progresses, the kidneys are but slightly enlarged, but still show a brown pigmentation. The surface may present a few scattered areas of hemorrhage and necrosis, as in the acute stage, interspersed with small depressed scars. The glomeruli are irregular, some being enlarged while others are much contracted. The tubular epithelium shows various degrees of degeneration, with a slight desquamation, or even patches of necrosis. Many tubules show regenerating epithelium with numerous mitotic figures. In these areas of regenerating tubular epithelium there is usually a pronounced interstitial infiltration of plasma cells and an increase in the connective tissue.

DISCUSSION

Clinically and pathologically, slight degrees of hematin intoxication cause only such alterations in urine and kidneys as are commonly observed in many febrile conditions. In more severe intoxications the irregularity and uncertainty of the effect on the kidneys is quite striking; some of these cases again show only the disturbances of a febrile state, while others present the clinical picture of an acute nephritis and a very few a definite hemoglobinuria. Throughout, the one constant feature is the albuminuria with casts and degenerative lesions in the kidneys — all probably the result of a slight toxic injury. The wide range of variety in urine and kidney lesions that may be superimposed on this common basis under constant experimental conditions suggests strongly the element of chance in the effect of hematin on the kidneys. This chance factor is operative through the blood-vessels. It has been demonstrated by physiological methods that hematin produces a marked dilatation of the splanchnic vessels[5] and causes injury to the vessel wall. Microscopically, the glomerular vessels in particular show these changes and, in addition, obstruction to circulation in many areas by hyalin thrombi or

5. Brown, Wade H., and Lovenhart, A. S.: Jour. Exper. Med., 1913, xviii. (In press.)

emboli. This occlusion of vessels seems to be the chance occurrence that determines the major part of the renal complications of hematin intoxication. The foci of tubular necrosis are but minute infarcts; the hemorrhage also is largely determined by vascular occlusion, although the injured and weakened vessel wall predisposes to hemorrhage.

A few kidneys have shown such a uniformity in the character and distribution of the lesions that one might be tempted to regard them as instances of acute nephritis, but in view of the great preponderance of lesions that are undoubtedly due to vascular occlusion and injury I am inclined to regard them all as of like nature.

No light has been thrown on the important subject of hemoglobinuria except that it is possible of production by intravenous injections of hematin in a very small percentage of cases. The hemoglobinemia resulting from hematin intoxication undoubtedly reaches the threshold value of a kidney showing vascular and tubular injury at a point below that of the normal kidney.

It is not possible to correlate closely the renal complications of hematin intoxication with those of human malaria. The disturbances of function and the lesions of the kidneys have been found much more pronounced in hematin intoxications than in comparable grades of malarial infection in man, while the predominance of the glomerular lesions observed in these experiments is not found in malaria. These differences are partly due to the difference in the concentration of hematin in the blood. It is believed, however, that the analogy is sufficiently close to render the facts disclosed by these experiments of value as a basis for a clearer comprehension of the mode of production of the renal complications of human malaria.

SUMMARY

1. Mild grades of hematin intoxication produce degenerative lesions in the kidneys and the urine shows a trace of albumin and casts.

2. Severe grades of hematin intoxication result in extensive dilatation, injury and occlusion of the renal vessels by hyalin thrombi or emboli, all of which are most pronounced in the glomerular vessels. Extensive degeneration and necrosis of tubular epithelium, hemorrhages and even anemic infarcts result from these vascular lesions. In such cases, the urine presents the characteristics of an acute nephritis.

3. In rare instances of severe hematin intoxication, hemoglobinuria may occur.

4. During the period of recovery from acute hematin poisoning and in chronic poisoning, the kidneys show both degenerative and proliferative processes. The glomerular tufts shrink and the capsular space and tubules are more widely dilated. The tubular epithelium shows degen-

eration and active regeneration with abundant mitotic figures. There are foci of round-cell infiltration and of connective tissue increase. There is also a slight diffuse increase in connective tissue. The urine is increased in amount and contains albumin with hyaline and granular casts.

5. The renal complications of hematin intoxication are believed to be due primarily to dilatation, injury and occlusion of renal vessels under the action of hematin.

ON THE USE OF PITUITARY EXTRACT IN OBSTETRICS*

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Although the first use of pituitary in obstetrical practice was made in England, but few papers dealing with its action have been published in English, while in Europe it has been so extensively used that a study of the large number of papers and cases now published enables one to formulate, with considerable precision, rules for its employment. This is especially true after one has given it, with success and failure, in one's own experience. This forms the justification for this paper.

In 1895, Oliver and Schaefer, in their series of experiments with organ extracts, followed up their papers on the striking effects produced by the injection of extracts of the adrenal by showing that extracts of the pituitary also produced a rise in blood-pressure. In 1898, Howell showed that it was the posterior lobe which possessed this property. This was confirmed by Schaefer and Vincent, and in 1901 Schaefer and Magnus showed that the extract of the infundibular portion increased very markedly the flow of urine. At this point the matter rested until 1906, when Dale, in the course of some observations on the action of ergot, noted that the extract of pituitary brought about a marked uterine contraction. This observation, however, was entirely lost sight of until Blair Bell and Hicks, led by some experiments which they had on hand, obtained from Dale some pituitary extract and produced with it marked contractions of the uterus in pregnant rabbits.

In consequence of the results obtained, Blair Bell was led to use it in some obstetrical cases. He presented his observations before the Liverpool Medical Institution, November, 1909, and his paper was published in December. In it he refers to its use in two cases of post-partum hemorrhage, and one case in which it was used in the expulsive stage of labor. In September, 1910, Aarons of Edinburgh read a paper at the International Congress in St. Petersburg, in which he reported success in its use in six cases of post-partum hemorrhage.

On June 25, 1909, Frankl-Hochwart and Fröhlich of Vienna reported on some experiments which they had made with pituitary extract. They were led to join forces in the study of the action of pituitary on the vegetative nervous system, as both of them had previous experience with

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the pituitary gland under pathological conditions, and from what was known of the pharmacological action of the extract they were led to infer that it would produce uterine contractions. In this respect their hopes were answered, and they found that pregnant rabbits showed a very marked increase in uterine movement after the administration of pituitary. The effect on blood-pressure was by no means marked, although it was found to be considerable when injected into dogs. They also observed, as they had expected, very marked effects on the urinary bladder and were able to show that it not only stimulated, but increased the irritability of both the nerves to the bladder and to the uterus.

In conclusion, they suggested that it should be used in obstetrics, and it was not long before the advice was acted on. In January, 1910, Foges and Hofstätter tried it in some cases to arrest post-partum bleeding. They showed that it had little effect on blood-pressure, but quite promptly brought about uterine contractions. They had several failures, three at least in which no effect whatever was produced.

Early in 1911 Hofbauer published the first series of cases in which it was used to increase labor pains. In his twelve cases the effects were always exceedingly striking, the movements coming on or increasing in force within a few minutes. He saw in no case any indication of tetanus uteri, but in several cases regular storms of contractions, bringing on a very rapid delivery. The publication of this exceedingly optimistic paper has led to pituitary being very widely used, and at least seventy-five papers dealing with the use of pituitary in labor have been published. An examination of the literature has disclosed a total of at least 1,650 cases in which it has been administered. A complete list of references is appended. The author has avoided, as far as possible, counting any case twice. The latest paper and the highest number of cases by any author being taken to include those previously reported.

My attention was attracted to the advantages of pituitary extract in obstetrics from the results seen in animal experimentation in the pharmacological laboratory. In the course of researches which have been in progress in this laboratory, and for demonstrations for student classes, pituitary has been used a considerable number of times to increase uterine movements. The accompanying curve (Fig. 1) is shown as very typical. As may be seen from this tracing, the uterine movements have been very markedly increased in both rate and amplitude, while the tone, as judged by the elevation of the line joining the bases of the waves, has not been raised, save at first where an irregular tetanus may be seen. In none of the cases in which it was employed has a true tetanus uteri, i. e., a very marked rise in tone with comparatively slight superimposed movements, been seen. Hahl and Malinowsky have recorded the variations in intra-uterine pressure with a bag, after the method of Westermarck, and in

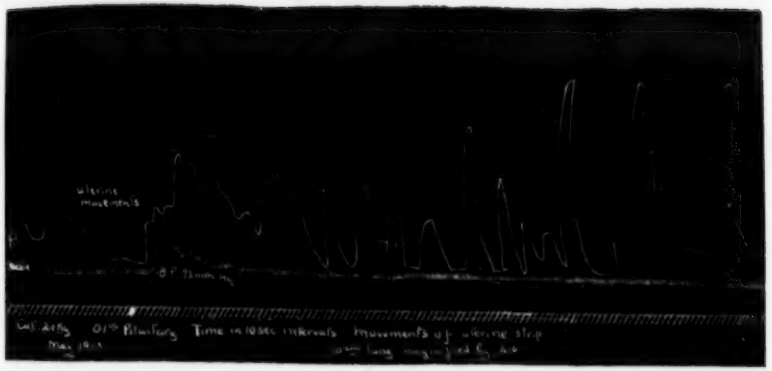

Figure 1

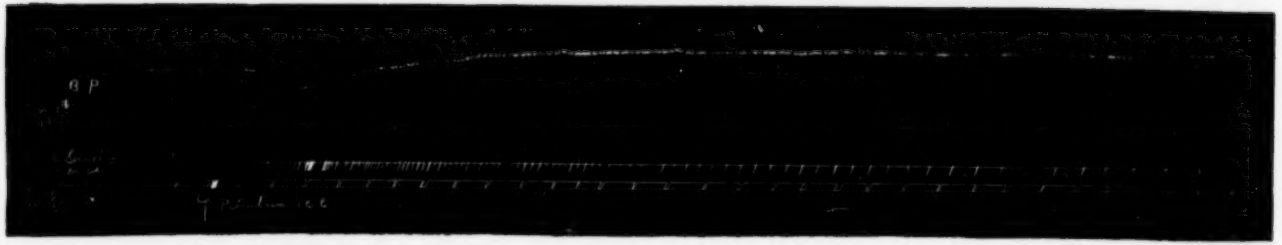

Figure 2

two cases reported by Hahl, there was some rise in tone, the movements being shorter and stronger, at shorter intervals and with increased intrauterine pressure; and Malinowsky shows a good tracing of a uterine tetanus. In other cases no increase in tone occurred, but only of movements. Such tetani are, however, not uncommon with ergot preparations, but apparently much less so with pituitary. Cases have, however, occurred. In one of the cases published by Rieck a tetanus evidently occurred, and is very well described by him. When it took place after the second injection, which was given six hours after the first injection, the patient stated that she felt the individual pains, but the examining hand could detect no contractions or relaxations.

Lieven also reports a case in which the uterine tetanus was so marked that the child's heart-rate fell steadily to 82, and there was a marked passage of meconium. Spaeth reports a similar case. He was, however, so unfortunate as to see the child die. Seitz and Roemer have each stated that they have also seen tetanus occur. Hamm, too, in one case which was brought to him after the child was dead and the patient had been in labor for a considerable period of time, injected pituitary, and regular movements set in. These, however, died away, and a second injection was given, which was followed in seven minutes by a tetanic constriction which lasted eight minutes, then regular movements for one or two hours, which gradually died away. A third injection produced another tetanus of eleven minutes' duration, then regular movements; and the fourth, tetanus of seventeen minutes, followed by regular movements, which led to delivery. He states that he has frequently seen the fetal heart-rate fall to 80; but in view of the rapidity of delivery in most cases, does not consider this dangerous. The above incident shows quite clearly, however, that the use of pituitary is not without some danger to the child, though danger to the mother seems very slight. In consequence, the fetal heart sounds should be, if it is at all possible, kept constantly under observation. This danger seems undoubtedly greatest when the drug is administered during the first stage.

A study of the literature has disclosed that the majority of observers, and especially those with the larger series of cases, have found that for the production of abortion, pituitary alone is insufficient. We might quote in this connection Schiffmann, who, out of seven cases, had three complete failures. Hell, Fischer, Nagy, Hirsch, Voigt, Merkel, Sellheim and Trapl have all pronounced against its use for this purpose.

Nor has it been of any great service in initiating premature labor. Trapl, Schiffmann, Fischer, Nagy, Hirsch, Voigt, Foges and Hofstätter, Merkel and Sellheim may be quoted in support of this statement. Nevertheless, successful cases have been observed in which one or two injections sufficed; and in the production of both abortion and premature labor, it

has proved in almost all cases a useful aid to other methods, such as dilatation by mechanical means.

Practically all observers are unanimous in declaring that it is of the greatest value in overcoming uterine weakness supervening after dilatation of the soft parts, and during the expulsive stage of labor. The effect, as a rule, is very prompt. The pains set in with great vigor in fifteen to twenty minutes or less, and are strong, rapidly leading to delivery; sometimes in a few minutes, and frequently within the hour. As mentioned above, pituitary has been used in at least 1,650 cases, but it is difficult to estimate in how many of these cases it was given during this phase of labor. Not more than a dozen failures during this stage are recorded. The total number of complete failures reported is less than fifty, and these failures are largely amongst those cases in which it was used for production of abortion or premature labor, without other means being employed, or post partum. Several observers have found that it produces movements that are so rhythmical and strong as to be of the greatest value in converting abnormal into normal positions. There is, of course, no general agreement as to what constitutes failure; and as details in all the longer series of cases are not given, it is impossible to express the results recorded in the literature in a more exact fashion. Hofbauer, in his last forty cases, reports no failures; Cahn, out of eighty-seven cases, three; Aubert, 15 per cent. of failures; Foges and Hofstätter, out of sixty-three cases, three failures. These figures seem very typical; but it must be noted that other observers, with as large a series or larger, say nothing of failure.

Its action in these cases is well illustrated by the following cases, selected from amongst those seen by me.

Case 1.—Primipara, 23 years of age Labor pains began about 3 a. m. When examined at 9 a. m. the cervix was soft and easily dilatable; but the pains were feeble, and during the course of the pain there did not appear to be any advance of the head, which was in the first position. The patient was seen several times through the day; and the soft parts, including the vagina and perineum, became gradually softened; but still the pains were very feeble, did not advance the head and caused the patient no distress whatever. At 4:30 p. m. 1 c.c. of infundibular extract, prepared by Messrs. Burroughs, Welcome & Co., was injected. Strong expulsive pains begain in five minutes. The contractions were regular and continuous. The head descended quickly and the child was born at 4:45 p. m. The uterus was well contracted. The placenta separated and was expelled in five minutes. Without pituitary, one would undoubtedly have used forceps in this case, as the patient was becoming rather tired, and would apparently have gone on for hours without delivering herself.

Case 2.—III-para, 25 years of age. This was the third pregnancy in three years, and she had not been feeling very strong during the latter weeks. Labor began at 9 p. m. The pains were only slight all night, but sufficient to dilate all the soft parts. They occurred every five minutes, but seemed to have no expulsive power. The patient did not appear to help herself very much, and as she was becoming tired, one had to consider interference. It was decided to try pituitary before forceps. One c.c. of the infundibular was injected at 7:20 a. m. In five

minutes strong rhythmical contractions occurred, which caused a rapid expulsion of the child in less than ten minutes. The placenta was expelled in five minutes.

Pfeifer, Sterne, Zinsser, Jaeger, Mory and Hamm have seen failure to produce delivery, owing to failure of the soft part to dilate. Hamm, indeed, saw physiological stricture develop in four cases where it was used to produce premature labor.

In the two cases of failure seen by me, the difficulty appeared to be that the soft parts did not become dilatable. No ill effects were produced, but forceps had to be applied.

CASE 3.—Primipara, 23 years of age. Labor began at 9 a. m. There were feeble pains all day, which became more severe at 10 p. m. The head was in the first position. Dilatation was progressive and the head descended. Then the pains became feeble, and had little expelling power. One c.c. of infundibular extract (B., W. & Co.) was injected. Stronger pains came on within twenty minutes, but the perineum did not relax, nor did the patient seem to help herself. Finally the forceps were applied and delivery effected. The uterus was well contracted, and the placenta was expelled within ten minutes.

CASE 4.—Primipara, 28 years of age. There were feeble pains throughout the day, which became more regular about 11 p. m. When seen at 2:30 a. m. the pains were occurring at about five-minute intervals. The patient was very nervous and did not help herself very much. The cervix was well dilated, and the pains were not very strong. One c.c. of infundibular extract was injected. In about twenty minutes the pains, as judged by the patient's sensations, became more severe; but the contractions were not appreciably quickened, nor did they advance the head, which was in the first position and fairly low down, although not bulging the perineum. After waiting some time, and as the patient was becoming tired, forceps were applied and delivery effected at 6 a. m. The uterus contracted well, and the placenta was expressed in ten minutes.

In this case the injection of pituitary extract did not seem to increase at all the expulsive power of the uterus; but there appeared to be a distinct lack of voluntary efforts on the part of the patient.

Several observers warn their colleagues not to employ pituitary after delivery of the child and before that of the placenta. Rieck reports a case of retention of the placenta. Hirsch, Voigt, Foges and Hofstätter, Rieck, Merkel, Seitz, Kroemer, have all found it not so valuable post partum; and several of these express a preference for ergot. In view of the greater frequency with which the latter in laboratory experience produces tetanus uteri, this is easily understood. Sellheim reports that out of twelve cases in which he used it, eleven were failures.

Several observers state that they have found it of no value in uterine atony; e. g., Anderes; and this condition probably explains the cases of complete failure to reawaken pains when they have prematurely ceased, such as reported by Zinsser (five out of sixty-five cases), Hamm (one case in forty).

In addition to the action of pituitary on the uterus, its action in increasing the movements of the urinary bladder are important. Jaschke and Franz have employed it as a postoperative tonic for this purpose;

and several of those who have used it in obstetrics have noted that the bladder was well emptied without the use of a catheter.

That in pituitary we have a drug which will increase the flow of milk, was shown by Mackenzie and confirmed by Ott and Scott, and may be illustrated by the curve produced in Figure 2. In this experiment the method employed was that of Mackenzie. A lactating cat was anesthetized, the skin removed from over two mammae, their nipples cut off, and the gland deeply incised. An injection of pituitary produced the prompt response shown in the tracing. In all, some 5 c.c. of milk were secreted. After an interval of thirty minutes a further injection produced an increased flow. The effect of an intravenous injection is evidently very marked, but brief. Whether single or even daily injections will lead to a permanent increase in milk production is not decided. The cases advanced in support of this view by Reynolds are not sufficiently numerous, nor were the observations recorded with sufficient care to be convincing.

CONCLUSIONS

1. Pituitary is of great value in cases of weakness in uterine movements after the soft parts are well dilated. Failure in these cases is rare, probably less than 1 per cent. The later in labor, but before delivery, the more striking the effect. The danger to the child and mother is very slight.

2. As an addition to some mechanical method, e. g., the Champetier de Ribes' bag, it is of great value in bringing on premature labor or abortion. In the former case it may be sufficient in itself, but there is some risk of tetanus of the cervix, or of the uterus, especially when repeated injections are required.

3. For delivery of the placenta its use is accompanied by the danger of tetanus uteri and retention.

4. In post-partum hemorrhage a considerable percentage of failures may be expected.

When a need for a uterine stimulant arises in cases conforming to the above indications, I believe that pituitary is of the greatest value, and will act as in Cases 1 and 2, which are typical of others in my experience.

I desire to express thanks to Dr. V. E. Henderson for the use of his laboratory, and invaluable assistance and criticism in preparing this paper.

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THE TREATMENT OF SYPHILITIC AFFECTIONS OF THE CENTRAL NERVOUS SYSTEM WITH ESPECIAL REFERENCE TO THE USE OF INTRA-SPINOUS INJECTIONS*

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Syphilis of the central nervous system is a manifestation of an infectious disease caused by a known parasite, against which we have at least two specific therapeutic agents. It is, therefore, amenable to treatment. The problems to be solved are: (1) How to recognize the condition before irreparable damage has been done to important structures; (2) the determination of the most efficient methods of application of our curative agents. The first can be solved only by careful clinical study and routine examination of the cerebrospinal fluid of all suspected patients. It is the purpose of this communication to present the results of an attempt to solve the second problem, viz., the determination of the best method of application of the curative agents.

The demonstration of the *Treponema pallidum* in general paralysis has definitely established the fact that in the so-called parasyphilitic nervous diseases there is active syphilis of the central nervous system. The cerebrospinal fluid in paresis and tabes shows qualitatively the same alterations as does the fluid in cerebrospinal syphilis. The usual changes occurring in the cerebrospinal fluid in these conditions are pleocytosis, with a predominance of mononuclear forms, and an increase in globulin. These abnormalities indicate the presence of a chronic inflammatory process in the cerebrospinal axis. A positive Wassermann reaction in the fluid indicates that the inflammation is syphilitic in nature. These abnormal constituents usually disappear more rapidly under specific treatment in involvement of the nervous system occurring early, than they do in such lesions occurring late in syphilis or in tabes or general paralysis, especially the latter. In late syphilis the spirochetes have established themselves in strongholds difficult to reach with our drugs. Such strongholds are the regions of poor vascularization, and in these the

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central nervous system is particularly rich. The *Treponema pallidum* has a predilection for lymph spaces. For practical purposes the perivascular lymphatics and the perineuronal spaces may be considered as lymph spaces which are not in direct communication with the general lymphatic system, but which are part of the subarachnoid space. Through them flows the cerebrospinal fluid. It has been well established that there is very little excretion of curative agents from the blood into the cerebrospinal fluid. Flexner and his co-workers have shown the necessity of directly introducing the therapeutic sera into the subarachnoid space in the various forms of purulent meningitis. The same principle no doubt holds true, to a considerable degree, in all forms of meningitis.

In dealing with syphilis of the central nervous system, we are, however, more fortunate than is the case in purulent meningitis. For here the introduction of our therapeutic agents into the general cirulation is of undoubted benefit. With mercury and iodids and with salvarsan intravenously much can be done, and in many patients all clinical signs and symptoms can be relieved. Efficient therapy, however, means more than the relief of symptoms; it involves also the eradication of all evidence of disease. This is more difficult. The changes occurring in the cerebrospinal fluid — pleocytosis, increased globulin and positive Wassermann reaction — must, as has been stated, be looked on as evidence of active syphilis of the central nervous system. No treatment can be considered adequate which does not result in the permanent disappearance of these abnormalities.

While rapid symptomatic improvement frequently follows the use of mercury and iodids, the relatively slight influence of these drugs on the abnormal constituents in the spinal fluid suggests at once the probability of subsequent relapse. In tabes the use of mercury seems frequently actually to increase the intensity of the symptoms. Salvarsan intravenously acts more rapidly than mercury, especially in reducing the degree of pleocytosis. To produce a disappearance of the increased globulin and to change the Wassermann reaction from positive to negative is, however, much more difficult.

The idea of intensifying the treatment of syphilis of the central nervous system by the introduction of salvarsan directly into the cerebrospinal fluid naturally suggests itself. To determine whether this might be safely done, we injected high dilutions of the drug in monkey-serum intraspinously into monkeys. Subsequent examination of the cerebrospinal fluid showed that the drug introduced in this form was too irritating to warrant its application to patients. Neosalvarsan injected intraspinously into monkeys was less irritating, but when injected into patients

produced symptoms too severe to warrant its continued use.[1] Serum, on the other hand, can be repeatedly injected into the subarachnoid space without demonstrable injury to the nervous tissue.

The blood-serum of patients treated intravenously with salvarsan has been shown to have definite therapeutic value when injected subcutaneously into patients with congenital and secondary syphilis.[2] We have been able to show that this serum also exerts a spirocheticidal action on the spirochetes of relapsing fever.[3] When the serum is allowed to act on the spirochetes *in vitro* and subsequently these are injected into mice, they do not develop in the mice as do untreated spirochetes or spirochetes on which normal serum has been allowed to act. Heating the serum to 56 C. increases the spirocheticidal action. We have also used the serum of syphilitic patients to make serum agar for the growth of *Treponema pallidum* after Noguchi's method. Cultures in media made with serum obtained before treatment grew practically as well as in that made with normal serum, while in media made with serum obtained an hour after intravenous injections of salvarsan the spirochetes grew more slowly or not at all. In media made with serum which was obtained at longer intervals after treatment, the growth approximated that in normal control tubes. These experiments indicate that the serum has the greatest inhibitory effect shortly after the injection of salvarsan.

All the above-mentioned experiments support the view that the serum of salvarsan-treated patients has a definite antispirochetal effect, both *in vitro* and *in vivo*. Such serums are ideal preparations for direct

1. Wechselmann (Deutsch. med. Wchnschr., 1912, xxxviii, 1446), reports that he had injected neosalvarsan intraspinously into two patients with general paralysis, and into two children with congenital lues, without any injurious effects. Marinesco (Ztschr. f. physik. u. diätet. Therap., 1913, xvii, 194), reports the results in thirteen patients, each of whom received 5 mg. neosalvarsan in 4 c.c. solution intraspinously. In ten of these severe unpleasant symptoms immediately appeared. Eight of them had permanent bladder disturbance. From this experience Marinesco advises strongly against the intraspinous injection of neosalvarsan. We are in complete accord with his conclusions. In the same communication Marinesco states that he had treated patients with syphilis of the central nervous system with serum obtained from patients with secondary syphilis after treatment with salvarsan. There were some beneficial results, but the work was not continued.

Robertson (Edinburgh Med. Jour., 1913, x, 428), reports on the intraspinous treatment of patients with general paralysis. For this treatment he used the serum of patients with secondary syphilis, which was withdrawn on the third day after a salvarsan injection, and also the paretics' own serum withdrawn one hour after the intravenous injection of salvarsan. Only a few treatments were given to each patient, but half of those treated showed some improvement.

2. Meirowsky and Hartmann: Med. Klin., 1910, vi, 1572; Plaut, H. C.: Deutsch. Med. Wchnschr., 1910, xxvi, 2237; Gibbs and Calthrop: British Med. Jour., 1911, i, 809.

3. To be published. Gonder, R. (Ztschr. f. Immunitätsforsch. Orig. 1912, xv, 257), reports on a similar spirocheticidal action of the blood of salvarsan-treated rats on the spirochetes of relapsing fever.

introduction into the subarachnoid space, and for the past two years we have been studying the results of the injection of such serums intraspinously into patients with syphilitic disease of the central nervous system.

TECHNIC OF SUBARACHNOID INJECTIONS

The technic that we have employed varies but little from that previously described,[4] and is as follows:

One hour after the intravenous injection of salvarsan 40 c.c. of blood is withdrawn directly into bottle-shaped centrifuge tubes, and allowed to coagulate, after which it is centrifugalized. The following day 12 c.c. of serum is pipetted off and diluted with 18 c.c. of normal saline. This 40 per cent. serum[5] is then heated at 56 C. for one-half hour. After lumbar puncture the cerebrospinal fluid is withdrawn until the pressure is reduced to 30 mm. cerebrospinal fluid pressure. The barrel of a 20 c.c. Luer syringe (which has a capacity of about 30 c.c.), is connected to the needle by means of a rubber tube about 40 cm. long. The tubing is then allowed to fill with cerebrospinal fluid so that no air will be injected. The serum is then poured into the syringe and allowed to flow slowly into the subarachnoid space by means of gravity. At times it is necessary to insert the plunger of the syringe to inject the last 5 c.c. of fluid. It is important that the larger part of the serum should be injected by gravity and if the rubber tubing is not more than 40 cm. long the pressure cannot be higher than 400 mm. Usually the serum flows in easily under even a lower pressure. By the gravity method the danger of suddenly increasing the intraspinous pressure to the danger point, such as might occur with rapid injection with a syringe, is avoided. Frequently there is a certain amount of pain in the legs, commencing a few hours after the injection. The pain is more often noticed in tabetics than in patients with cerebrospinal syphilis. It can usually be controlled by means of phenacetin and codein. Occasionally morphin is required.

The therapeutic effect of this form of treatment is illustrated in the tables, where the treatment, Wassermann reaction in the blood and in the cerebrospinal fluid, the cell count and globulin in the fluid of each patient are given. In some of the tables, the Wassermann reaction in the cerebrospinal fluid is indicated with two different antigens. Prior to January, 1913, fetal liver extract was used as antigen. It was then found that an alcoholic extract of human heart, to which 0.4 per cent. cholesterin had been added, was a much more sensitive reagent.[6] In order not to confuse the results it was considered wise to continue the use of the liver antigen in those cases in which it had previously been used, and to add the comparative reactions with the cholesterin-heart antigen. Unless otherwise designated in the notes after January, 1913, a completely negative reaction indicates that it was negative with the cholesterin-heart as well as with the liver antigen. In the cerebrospinal fluid column, the amount of fluid which gave complete fixation is indi-

4. Swift, H. F., and Ellis, A. W. M.: New York Med. Jour., 1912, xcvi, 53.

5. In patients who do not have reactions following the injection of 40 per cent. serum the strength is at times increased to 50 or 60 per cent., or even stronger.

6. Walker, I. C., and Swift, H. F.: Jour. Exper. Med., 1913, xviii, 75.

cated.[7] The quantitative titration of the strength of reaction has been used since November, 1911. Previous to that time, only 0.1 c.c. of fluid was used. The titration of the strength of the reaction furnishes a fair index of the effect of treatment. In this report we wish to lay more emphasis on the improvement in the condition of the cerebrospinal fluid than on the clinical improvement, because it is entirely objective and probably gives us a better idea of the actual effect of our therapeutic measures.

TABLE 1.—No. 85. C. L. V. Age 35. Tabes Dorsalis Six Years. Syphilis Sixteen Years. Result: Improved

Date, 1910	Blood Wassermann Reaction	Cerebrospinal Fluid			Treatment	
		Cells per c.mm.	Noguchi Globulin	Wassermann Reaction, c.c. Spinal Fluid	Intravenous Salvarsan, gm.	Intraspinous Serum
Nov. 10 to. 1911	++	...	....	.....	‡	.....
Jan. 10 ...	+	...	....	.....	.....	.....
Jan. 16 ...	±	125	++	0.1 —	0.2	.....
Jan. 25 ...	+	...	....	.....	0.3	.....
Feb. 8	+	...	....	.....	0.36	.....
Feb. 13 ...	....	150	±	0.1 —	.....	.....
Feb. 15 to..	±	...	....	.....	†	.....
Aug. 20 ...	....	...	....	.....	†	.....
Aug. 21 ...	±	63	+	0.1± ?	†	.....
Aug. 26 to.	....	...	....	.....	.....	.....
Sept. 22 ..	± ?	...	....	.....	§0.2	.....
Sept. 22 ..	.....	26	±	0 1 —	.....	.....
Nov. 24 ..	....	...	....	.....	0.2	.....
Dec. 1* ...	± ?	50	+	0.5 ±	0.2	18 c.c. of 50%
Dec. 11 ...	±	20	+	.....	0.2	30 c.c. of 50%
Dec. 18 ...	±	20	±	0.5 ±	0.2	20 c.c. of 40%
1912						
Jan. 5	—	12	±	0.5 +±	0.3	30 c.c. of 40%
Jan. 12 ...	—	4	—	0.5 ±	0.3	30 c.c. of 50%
Jan. 15 ...	—	...	....	.....	0.2	.....
Jan. 20 ...	....	5	—	0.5 ± ?	.....	.....
July 9	—	2	—	0.5 —	.....	.....
Sept. 10 ..	—	1	—	0.5 —	.....	.....
1913						
May 15 ..	—	3	±	0.5 —	.....	.....

* Where both intravenous and intraspinous treatment were used the date of the intravenous injection is indicated. The intraspinous injection was given on the following day.

† Mixed treatment by mouth.

‡ Sixteen injections $HgCl_2$ intramuscularly.

§ Five injections given.

7. In performing the reaction with the cerebrospinal fluid 0.5 c.c., 0.4 c.c., 0.3 c.c., 0.2 c.c., 0.1 c.c. and 0.05 c.c. fluid are used. As we employ one-half quantities of all the reagents as compared with the amounts originally recommended for the Wassermann reaction, these quantities therefore correspond to 1.0 c.c., 0.8 c.c., 0.6 c.c., 0.4 c.c., 0.2 c.c. and 0.1 c.c., respectively.

CASE REPORTS

CASE 85.—C. L. V., a well marked tabetic, who had had symptoms for about six years, lately becoming more marked. He had lightning pains, difficulty in walking, girdle sensation about the abdomen, and Charcot joint in the right hip. He had received practically no antisyphilitic treatment prior to November, 1910. In the first year of treatment he received sixteen intramuscular injections of mercuric chlorid, nine intravenous injections of salvarsan, a total of 2 gm., and six months' mixed treatment by mouth. The result of this was to reduce the pleocytosis from 125 to 50 per cm. The effect on the Wassermann reaction in the cerebrospinal fluid was not determined, because an insufficient quantity of fluid was employed. After the institution of intraspinous treatment with his own serum the fluid became normal in six weeks. During this period a total of 1.4 gm. salvarsan was given in six intravenous injections and five intra-spinous injections, a total of 60 c.c. of undiluted serum.[6] For over a year the fluid has

TABLE 2.—No. 213. O. W. A. AGE 29. TABES DORSALIS TWO AND ONE-HALF YEARS. SYPHILIS NINE YEARS. RESULT: IMPROVED

Date, 1911	Blood Wass. Reaction	Cerebrospinal Fluid				Treatment	
				Wassermann Reaction			
		Cells per c.mm	Noguchi Globulin	Liver Antigen, Spinal Fluid c.c.	Cholesterin Heart Antigen, Spinal Fluid, c.c.	Intravenous Salvarsan, gm.	Intraspinous Serum
June 23 to	++	...	....	.....	.....	.....	.....
Aug. 2	++	...	....	.....	.....	5 x 0.2	.....
Aug. 5	....	110	—	0.1 ±	.....	.....	.....
Aug. 10 to	++	...	....	.....	.....	.....	.....
Oct. 20 ...	+	...	....	.....	.....	4 x 0.2	.....
Oct. 27 ...	+	75	—	0.1 ++	.....	0.2	25 c.c. of 40%
Nov. 3 ...	++	...	....	.....	.....	0.2	.....
Nov. 21 ...	++	...	....	0.1 ++	.....	0.2	20 c.c. of 50%
Dec. 12 ...	++	20	±	0.1 ++	.....	0.2	30 c.c. of 70%
Dec. 19 ...	++	19	±	0.2 ++	.....	0.2	25 c.c. of 40%
1912							
Jan. 5	+	22	—	0.1 ++	.....	0.3	30 c.c. of 50%
Jan. 12 ...	+	8	—	0.3 ++	.....	0.3	30 c.c. of 50%
Jan. 19 ...	+	10	—	0.3 ++	.....	0.3	30 c.c. of 50%
Jan. 25 ...	+±	8	—	0.2 ++	.....	0.3	30 c.c. of 50%
Feb. 1	....	5	—	.....	.....	0.3	25 c.c. of 40%
Feb. 8	++	7	—	0.2 ++	.....	0.3	30 c.c. of 50%
April 11 ..	+	2	—	0.3 ++	.....	0.3	30 c.c. of 40%
April 18 ..	±	...	....	.....	.....	0.3	.....
April 25 ..	++	10	—	0.3 ++	.....	0.3	30 c.c. of 40%
May 2	++	8	—	0.3 ++	.....	0.3	30 c.c. of 40%
May 16 ...	+	10	—	0.3 ++	.....	0.3	30 c.c. of 40%
1913							
Feb. 22 ...	++	1	—	0.4 ++	0.3 ++	*0.75	30 c.c. of 40%

* Neosalvarsan.

6. In the tables the amount of diluted serum which was injected at each treatment is shown, while in the abstracts in the text the total amount of undiluted serum is given.

remained normal and the patient has been practically free from pain. The Charcot joint has prohibited his return to his usual work, although he goes about very well. There has been no advance in his tabes.

CASE 213.—O. W. A. had a rapidly progressing tabes of two and a half years' duration. The most marked symptoms were ataxia, pain and hyperesthesia in the lumbar region. In the first four months of treatment a total of 1.8 gm. of salvarsan was given in nine intravenous injections, which produced a drop in cells from 125 to 75, but had no effect on the Wasserman reaction in the cerebrospinal fluid. On the institution of intraspinous injection of patient's own serum there was an immediate drop in the number of cells. In the next seven months he received 4.3 gm. salvarsan in sixteen intravenous injections and fourteen intraspinous injections, a total of 184 c.c. undiluted serum. Under this treatment the cells were reduced to practically normal in number, and the Wassermann reaction in the cerebrospinal fluid was much reduced in strength. Because the patient felt so much better he neglected to report for nine months, during which time the fluid remained practically the same.

A marked beneficial effect was noted in both these patients when the intraspinous treatment was instituted, but the objection might be advanced that the improvement was due to the continuation of the salvarsan intravenously. To determine the effect of the intraspinous treatment alone, several patients were treated intraspinously with the serum from other patients who had received intravenous injections of salvarsan.

TABLE 3.—No. 557. C. H. L. TABES DORSALIS TEN YEARS. SYPHILIS THIRTY YEARS. RESULT: IMPROVED

Date, 1911	Blood Wass. Reaction	Cerebrospinal Fluid			Treatment	
		Cells per c.mm.	Noguchi Globulin	Wassermann Reaction, c.c. Spinal Fluid	Intravenous Salvarsan, gm.	Intraspinous Other Patients' Serum
Oct. 25....	—	42	++	0.1 ++	.....	.....
Oct. 30....	..	41	++	0.1 ++	.....	25 c.c. of 40%
Nov. 6.....	..	40	++	0.1 ++	.....	20 c.c. of 40%
Nov. 18....	..	50	++	0.2 ++	.....	22 c.c. of 40%
Dec. 3.....	—	18	++	0.2 ++	.....	26 c.c. of 50%
Dec. 12....	..	17	++	0.1 ++	.....	30 c.c. of 50%
Dec. 28....	..	15	++	0.4 ++	.....	25 c.c. of 40%
1912						
Jan. 6.....	..	15	+	0.1 ++	.....	25 c.c. of 40%
Jan. 12....	..	10	++	0.2 ++	.....	30 c.c. of 50%
Jan. 23....	..	14	++	0.5 ±?	.....	26 c.c. of 50%
Jan. 31....	..	16	+	0.5 ±	.....	30 c.c. of 50%
Feb. 7.....	..	17	+	0.5 +±	.....	30 c.c. of 50%
March 4...	—	15	+	0.5 +±	.....	.....
April 10...	..	10	±	0.5 ±	.....	.....
April 16 to	—	..	....	.....	.....	.....
May 14....	—	..	....	.....	*0.3	.....
May 14....	..	19	+	0.5 ±	.....	.....
Sept. 24....	—	8	±	0.5 —	.....	.....
1913						
March 28..	—	5	—	0.5 —	.....	.....

* Five injections given.

CASE 557.—C. H. L., a man 50 years of age, with fairly well marked tabes dating back ten years since the onset. He had had marked gastric disturbances for the past three years, so that he had lost much weight and had been incapacitated for several months. In three and one-half months he received eleven intraspinous injections, a total of 130 c.c. undiluted serum, which was obtained from other patients. This treatment resulted in the reduction of all the abnormal constituents in the fluid nearly to normal. Then 1.5 gm. salvarsan in five intravenous injections was given with practically no effect on the fluid. The patient was so much improved that he returned to work and received no further treatment. At the time of the last examination of the fluid, nearly one year after treatment was discontinued, his spinal fluid was entirely normal. The tabetic process has shown practically no advance since the treatment was started.

TABLE 4.—No. 675. H. G. G. AGE 42. TABES DORSALIS TWO AND ONE-HALF YEARS. SYPHILIS DENIED. RESULT: IMPROVED

Date, 1912	Blood Wassermann Reaction	Cerebrospinal Fluid			Treatment
		Cells	Noguchi Globulin	Wass. Reaction c.c. Spinal Fluid	Intraspinous Other Patients' Serum
March 18....	±	35	±	0.3 ++	.....
May 2.....	—	..	..	.....	.....
May 5.....	..	41	+	0.3 ++	30 c.c. of 40%
May 14.....	..	24	±	0.4 ++	30 c.c. of 40%
May 21.....	..	17	±	0.4 ++	30 c.c. of 40%
June 10.....	—	..	..	.....	.....
June 20.....	..	14	±	0.5 ++	30 c.c. of 40%
June 27.....	..	33	±	0.5 ++	30 c.c. of 40%
July 9.....	..	12	±	0.5 ++	30 c.c. of 40%
July 21.....	..	35	+	0.5 ++	30 c.c. of 40%
July 30.....	—	..	..	.....	.....
Sept. 19.....	..	14	—	0.5 ±	30 c.c. of 40%
Oct. 1.....	..	8	—	0.5 —	30 c.c. of 40%
Oct. 19.....	..	7	—	0.5 —	30 c.c. of 40%
1913					
Jan. 5.....	—	5	—	0.5 —	.....

Died of lobar pneumonia. January 5. Autopsy showed typical tabes.

CASE 675.—H. G. G., a man 42 years of age, who had well marked tabes of two and one-half years' duration. He had never received any antisyphilitic treatment previous to his admission. In five and one-half months he was given ten intraspinous injections, a total of 120 c.c. of undiluted serum. After eight treatments the cerebrospinal fluid was normal. About three months after his last treatment he died of lobar pneumonia. The cerebrospinal fluid taken immediately after death was normal. Autopsy revealed changes in the cord typical of tabes dorsalis. The symptomatic improvement, as well as the return to normal of the cerebrospinal fluid, were most striking. This patient received no intravenous treatment at any time.

TABLE 5.—No. 565. V. H. Age 42 Years. Tertiary Syphilitic Meningitis (Radiculitis). Syphilis Nine Years Ago

Date, 1912	Blood Wassermann Reaction	Cerebrospinal Fluid			Treatment
		Cells	Noguchi Globulin	Wass. Reaction c.c. of Spinal Fluid	Intraspinous Other Patients' Serum
April 5.....	+±	124	++	0.1 ++	.....
April 10.....	....	64	++	0.1 +±	30 c.c. of 40%
April 19.....	....	17	+	0.2 ++	30 c.c. of 40%
April 28.....	....	30	+	0.2 ++	30 c.c. of 40%
May 7.....	....	23	+	0.2 ++	30 c.c. of 40%
Sept. 12*....	....	4	—	—	.....

* Report of findings on September 12 furnished by Neurological Institute.

Case 565.—V. H., a man 42 years old, who had pains referable to dorsal root irritation for three years. One year before admission he had received two intravenous injections of salvarsan followed by a course of mercury inunctions and considerable potassium iodid by mouth. Before this treatment was started he had 94 cells per c.mm. in the cerebrospinal fluid. Our first examination showed 124 cells, a heavy globulin and a strongly positive Wassermann reaction. He then had 48 c.c. of serum in four intraspinous injections. Four months later he was examined in another hospital and his spinal fluid reported as normal. Unfortunately, the patient would not continue under observation. The symptomatic improvement was less marked than the improvement in the pathological cerebrospinal fluid.

TABLE 6.—No. 485. C. M. Age 48. Tabes Dorsalis One and One-Half Years. Syphilis Nineteen Years

Date, 1912	Blood Wass. Reaction	Cerebrospinal Fluid			Treatment	
		Cells per c.mm.	Noguchi Globulin	Wassermann Reaction c.c. Spinal Fluid	Intravenous Salvarsan, gm.	Intraspinous Serum
Jan. 26....	—	130	++	0.5 ++	.....	.....
Jan. 30....	—	90	++	0.5 +	0.2	30 c.c. of 50%
Feb. 6.....	—	40	++	0.5 ±	0.3	.....
Feb. 13....	—	26	++	0.5 —	0.3	25 c.c. of 40%
Feb. 20....	—	24	++	0.5 —	0.3	30 c.c. of 40%
April 3....	—	9	+	0.5 —	0.3	20 c.c. of 40%
April 17...	—	...	.....	.....	0.3	.....
May 6.....	—	6	+	0.5 —	0.3	30 c.c. of 40%
June 20...	—	8	+	0.5 —	0.3	30 c.c. of 40%
July 18....	—	4	±	0.5 —	0.3	30 c.c. of 40%
Aug. 15....	—	4	±	0.5 ±?	0.3	30 c.c. of 40%
Sept. 12...	—	2	±	0.5 ±?	0.4	30 c.c. of 40%
Oct. 29....	—	...	....	.....	*0.9	.....
Nov. 19....	—	4	+	0.5 —	.....	.....
Dec. 17....	—	...	....	.....	*0.9	.....
1913						
April 9....	—	3	+	0.5 —	0.5	.....

* Neosalvarsan.

These three cases illustrate very well that definite and marked change in the condition of the cerebrospinal fluid can be brought about by the intraspinous injection of serum alone, since none of these last three patients had had any other form of treatment. As there is also a definite effect from intravenous injection of salvarsan, it would seem that the combination of these two forms of treatment would produce a more rapid effect than either alone. This is well illustrated by the next three cases.

CASE 485.—C. M., a man aged 48 years, with moderately advanced tabes of two years' duration. He had transient diplopia seven years ago. In eight months patient was given 3.3 gm. salvarsan in eleven intravenous injections, and nine intraspinous injections, a total of 109 cubic centimeters of his own serum. After the fourth intraspinous treatment the fluid was practically normal except for a moderate increase in globulin which has persisted up to the present time.

TABLE 7.—No. 893. F. B. AGE 34. TABES DORSALIS TWO YEARS. SYPHILIS THIRTEEN YEARS. RESULT: IMPROVED

Date, 1912	Blood Wass. Reaction	Cerebrospinal Fluid			Treatment	
		Cells per c.mm.	Noguchi Globulin	Wassermann Reaction, c.c. Spinal Fluid	Intravenous Neosalvarsan, gm.	Intraspinous Serum
Oct. 18.....	+	40	±	0.5 +	0.75	.....
Oct. 28.....	++	...	..	.....	0.45	.....
Nov. 2.....	..	57	+	0.5 +±	.....	.....
Nov. 4.....	++	47	+	0.5 ++	0.9	30 c.c. of 40%
Nov. 12.....	....	13	+	0.5 —	0.9	30 c.c. of 40%
Nov. 18.....	....	...	..	.....	0.9	.....
Nov. 25.....	++	...	..	.....	0.9	.....
Dec. 2.....	++	10	+	0.5 —	0.9	30 c.c. of 40%
Dec. 16.....	....	...	..	.....	0.9	.....
Dec. 17.....	....	2	±	0.5 —	.....	.....
1913	*			*	.....	.....
Jan. 25.....	....	7	±	0.5 —	.....	.....
Feb. 8.....	++	5	±	0.5 —	0.9	30 c.c. of 40%
March 14..	++	...	..	.....	0.75	.....
March 28 to	++	...	..	.....	.....	.....
April 25...	++	...	..	.....	† 3	.....
April 25...	++	3	±	0.5 —	.....	.....

* Cholesterin heart antigen.

† Salvarsan. Three injections given.

CASE 893.—F. B., a man 34 years of age, who had early tabes, suffering chiefly from shooting pain in the legs for two years, and slight unsteadiness in gait for six months. In four months he received 8.2 gm. neosalvarsan and four intraspinous injections, a total of 48 c.c. of his own serum. The increase in the intensity of the Wassermann reaction after the treatment was started is worthy of note. The sudden drop in cells and the disappearance of the Wassermann reaction occurred after the first intraspinous injection. After the fluid was reduced to normal except for a slight increase in globulin, the treatment was continued intravenously because of the persistence of the Wassermann reaction in the blood serum. This case illustrates the point which we have noticed, that

if, before treatment was started, large quantities of the fluid are required to obtain a positive Wassermann reaction, the reaction disappears fairly rapidly under treatment.

TABLE 8.—No. 605. F. K. Age 27. Cerebrospinal Syphilis. Duration (?) Previous Treatment None. Result: Improved

Date, 1912	Blood Wass. Reaction	Cerebrospinal Fluid			Treatment	
		Cells per c.mm	Noguchi Globulin	Wassermann Reaction c.c. Spinal Fluid	Intravenous Salvarsan, gm.	Intraspinous Serum
April 25...	++	59	+	0.2 ++	0.3	30 c.c. of 40%
May 2.....	++	...	..	0.3 ++	0.3	30 c.c. of 40%
May 9.....	+	...	..	.....	0.3	.....
May 23....	+	14	+	0.3 ++	0.3	30 c.c. of 40%
June 13....	+	5	±	0.3 ++	0.3	30 c.c. of 40%
June 20....	+	6	±	0.4 ++	0.3	30 c.c. of 40%
July 18....	±	2	±	0.5 ++	0.3	30 c.c. of 40%
July 26....	..	...	..	.....	0.3	.....
Aug. 15....	±	2	±	0.4 ++	0.3	30 c.c. of 40%
Sept. 12....	±	6	±	0.4 ++	0.4	30 c.c. of 40%
Oct. 3.....	±	...	..	.....	0.6	.....
Oct. 17.....	—	...	..	.....	0.6	.....
Nov. 2.....	..	7	—	0.5 ++	0.6	.....
Nov. 22....	+	...	..	.....	0.6	.....
Nov. 29 ..	..	...	..	.....	†0.9	.....
Dec. 12....	—	8	—	0.5 ++	†0.9	.....
1913	*			*		
Jan. 3.....	++	4	—	0.5 ++	†0.9	30 c.c. of 40%
Jan. 31....	++	3	—	0.5 +±	†0.9	30 c.c. of 40%
Feb. 28....	+±	4	—	0.5 +	†0.9	30 c.c. of 40%
April 4....	+±	...	..	.....	0.6	.....
April 25...	+	2	—	0.5 —	0.5	30 c.c. of 40%

* Cholesterin heart antigen.

† Neosalvarsan.

Case 605.—F. K., a man 27 years of age, who was admitted because of ophthalmoplegia interna of the left eye. He denied syphilis and had not taken anti-syphilitic treatment. His physical examination was normal except for one dilated pupil. In one year he received twenty intravenous injections, a total of 6.6 gm. of salvarsan and 4.5 gm. neosalvarsan, and 144 c.c. of his own serum in twelve intraspinous injections. At the end of this time the cerebrospinal fluid became normal and there was a distinct diminution in the intensity of the Wassermann reaction in the blood-serum.

This is the type of case which Nonne and Erb have described as usually going on to tabes or paresis. The distinct changes which were first found in the cerebrospinal fluid indicate that the patient was suffering from an active syphilis of the central nervous system, although he presented as physical signs only one dilated pupil. It seems reasonable to suppose that the treatment with the resulting return to normal of the cerebrospinal fluid has resulted in an arrest of the syphilitic process.

Probably by careful clinical examination, as well as examination of the cerebrospinal fluids, many cases of tabes could be detected in this early stage and arrested by applying intensive and prolonged treatment. It is worthy of note that many of the tabetics give a history of diplopia or other transient eye disturbances several years before the onset of any other symptoms. It is important to recognize the disease in this early stage in order that it may not be allowed to progress until marked nerve degeneration has occurred.

TABLE 9.—No. 152. O. F. Age 32. Tabes Dorsalis Three and One-Half Years. Syphilis Ten Years. Result: Improvement, Relapse, Improvement

Date, 1911	Blood Wass. Reaction	Cerebrospinal Fluid				Treatment	
				Wass. Reaction			
		Cells per c.mm.	Noguchi Globulin	Liver Antigen c.c of Spinal Fluid	Cholesterin Heart Antigen c.c. of Spinal Fluid	Intravenous Salvarsan, gm.	Intraspinous Serum
April 4 to.	++	27	++	0.1 —	.....	.....	.....
May 18....	+	...	....	.....	.....	5 x 0.2	.....
May 19....	....	39	++	0.1 +	.....	.....	.....
Oct. 13....	+	...	....	.....	.....	0.2	.....
Oct. 20....	±	9	+	0.1 ±	.....	0.2	17 c.c. of 40%
Oct. 27 to..	....	...	....	.....	.....	.....	.....
Nov. 17...	....	...	....	.....	.....	4 x 0.2	.....
Nov. 20...	....	10	+	.....	.....	.....	.....
1912							
April 9....	—	14	±	0.2 ++	.....	0.3	30 c.c. of 40%
April 15...	....	...	....	.....	.....	0.3	.....
April 23...	±	3	+	0.2 ++	.....	0.3	30 c.c. of 40%
April 30...	±	...	....	.....	.....	0.3	.....
May 7.....	±	6	+	0.2 ++	.....	0.3	30 c.c. of 40%
Sept. 23...	±	19	+	0.3 ++	.....	§0.9	.....
Oct. 8.....	±	...	....	... 0	.....	§0.9	.....
1913	*			....	.....	.....	.....
Jan. 31....	+±	70	+	0.2 ++	0.1 ++	§0.75	.....
Feb. 7.....	+±	57	±	0.2 ++	0.2 ++	§0.9	30 c.c. of 40%
Feb. 14....	+±	28	±	0.3 ++	0.2 ++	§0.9	30 c.c. of 40%
Feb. 28....	++	17	±	0.3 ++	0.2 ++	§0.09	30 c.c. of 40%
March 14..	+	14	—	0.2 ++	0.2 ++	§0.75	30 c.c. of 40%
March 27..	++	7	+	0.5 ++	0.3 ++	§0.75	30 c.c. of 40%
April 9....	+±	7	+	0.5 ±	0.3 ++	§0.9	30 c.c. of 40%

* Cholesterin heart antigen. § Neosalvarsan.

Case 152.—O. F., a man 32 years of age, who illustrates both the result of intraspinous treatment and the necessity of continuing some form of treatment until the cerebrospinal fluid is normal. He had a well marked tabes of three and one-half years' duration, with severe shooting pains. Previous to his admission he had received very little anti-syphilitic medication. In the first year he received 2.2 gm. salvarsan in eleven intravenous injections, which

resulted in slight reduction in the degree of pleocytosis, a moderate reduction in globulin, and as far as could be determined, little if any effect on the Wassermann reaction in cerebrospinal fluid. Then in one month's time he had 1.5 gm. salvarsan in five intravenous injections plus three intraspinous injections, a total of 36 c.c. of his own serum. This resulted only in a reduction of the pleocytosis. For the next eight months he refused to have systematic treatment. In January, 1913, the pains became more severe and about the end of the month he developed diplopia. Examination of the cerebrospinal fluid revealed a more marked pleocytosis than on any previous examination. In the next two months systematic treatment consisting of seven intravenous injections, a total of 5.8 gm. neosalvarsan, and six intraspinous injections of his own serum, a total of 72 c.c., resulted in a reduction in cells to nearly normal, and a marked decrease

TABLE 10.—No. 113. W. L. S. Age 52. Tabes Dorsalis Seven Years. Syphilis Twenty-Three Years. Improvement. Relapse

Date, 1911	Blood Wass. Reaction	Cerebrospinal Fluid			Treatment	
		Cells	Noguchi Globulin	Wass. Reaction, c.c. Spinal Fluid	Intravenous Salvarsan, gm.	Intraspinous Serum
March 7...	++	72	++	0.1 —	.....	.....
March 11..	++	...	....	.....	0.35	.....
March 28..	++	27	++	0.1 —	0.5	.....
April 4....	+	...	....	.....	†	.....
May 22....	++	...	....	.....	†	.....
Oct. 5.....	++	...	....	.....	†	.....
Oct. 7 to...	±	...	....	.....	.....	.....
Nov. 4.....	±	...	....	.....	‡0.2	.....
Nov. 5.....	....	36	+	0.1 —	.....	.....
1912						
Jan. 5.....	+	24	++	0.4 ++	0.3	30 c.c. of 40%
Jan. 13....	+	30	+	0.4 ++	0.3	28 c.c. of 40%
Jan. 21....	++	10	++	0.4 ++	0.3	20 c.c. of 50%
Jan. 27....	+	8	++	0.5 ++	0.3	25 c.c. of 40%
Feb. 3.....	+	28	+	0.4 ++	0.3	30 c.c. of 50%
Feb. 10....	++	5	++	0.4 ++	0.3	30 c.c. of 50%
March 16..	+	9	+	0.5 +	0.3	30 c.c. of 40%
March 23..	+	3	+	0.4 ++	0.2	30 c.c. of 40%
April 6....	±	3	±	0.5 —	0.2	30 c.c. of 40%
April 13...	+	3	±	0.5 —	0.3	.....
May 27 to July 8.....				.....	§	.....
Sept. 13...	+	2	±	0.5 —	¶0.9	.....
Sept. 21....	±	...	....	.....	¶0.9	.....
Nov. 23....	....	6	+	0.5 —	.....	.....
1913	*			*	.....	.....
Jan. 25....	++	9	±	0.5 —	¶0.6	.....
Feb. 8.....	+±	...	....	.....	¶0.6	.....
Feb. 22....	++	...	....	.....	¶0.6	.....
March 8....	+±	...	....	.....	¶0.75	.....
March 22...	+±	...	....	.....	¶0.75	.....
April 5....	++	7	+	0.4 +±	¶0.6	.....

* Cholesterin heart antigen. ‡ Five injections given.

† Mixed treatment by mouth.

§ Six injections Hg. salicylate intramuscularly.

¶ Neosalvarsan.

in the intensity of the Wassermann reaction in the cerebrospinal fluid. At the same time the pains and diplopia disappeared. The symptomatic relapse and the increase in the signs of the active pathological process as shown by the cerebrospinal fluid in the patient is in striking contrast to the subsequent course of the disease in those patients whose cerebrospinal fluid had been rendered normal by treatment.

CASE 113.—W. L. S., a man 52 years of age, who had a moderate tabes of seven years' duration. The principal symptom was shooting pain. In the first ten months of treatment he had seven intravenous injections of salvarsan, a total of 1.8 gm., and six months mixed treatment. This resulted in a reduction in cells from 72 to 36 per c.mm., and a moderate decrease in globulin. In the next three months he received 2.5 gm. salvarsan in nine intravenous injections and nine intraspinous injections of his own serum, a total of 109 c.c. At the end of this course of treatment the cerebrospinal fluid was normal except for a trace of globulin. Because of the persistence of a positive Wassermann reaction in the blood, intramuscular injections of mercury were given, but could not be tolerated long. Occasional lumbar puncture showed that the cells were increasing slightly in number, and systematic intravenous treatment with neosalvarsan was given. After five treatments, a total of 3.3 gm., it was found that the spinal fluid again showed a positive Wassermann reaction with 0.4 c.c. This returning reaction cannot be attributed to the increased sensitiveness of the cholesterin-heart antigen, because the reaction was negative in January with the same antigen. This patient has shown less symptomatic improvement than any of our other patients. After a course of treatment there would be some decrease in pain and periods of a week or more without pain, but no permanent relief has been obtained. The return of the positive Wassermann reaction in the cerebrospinal fluid seems to indicate that the syphilitic process is still active in some part of the cerebrospinal axis. The disappearance of the reaction under combined intraspinous and intravenous treatment, and its reappearance under intravenous treatment alone, seems to point to the advantage of the combined method in this patient at least.

DISCUSSION

The cases cited above are only examples of the various groups of patients whom we have under treatment. From the experience of the past two years we now feel that 0.2 gm. or 0.3 gm. salvarsan is too small a dose, and that neosalvarsan given in the same relative amounts as salvarsan is much inferior to salvarsan. At present we are giving full doses of salvarsan—0.45 gm. to 0.5 gm.—every two weeks, and in addition intraspinous injections of 30 c.c. of 40 per cent. serum, until the cerebrospinal fluid shows a normal cell count and a negative Wassermann reaction. Under this plan of treatment many of the cases are showing more rapid improvement than did those treated a year or more ago, illustrated in these tables. Unfortunately, our experience with general paralysis is too limited to give definite results. One patient with early paresis and one or two others who are border-line cases between tabes and paresis have shown a rapid decrease in pleocytosis and a moderate decrease in the globulin, but the Wassermann reaction has been slower in showing a response. When one considers the anatomical difficulties in reaching the myriads of spirochetes that are present in the brain in this condition, he is impressed with the necessity of prolonged and vigorous treatment.

Another point brought out by the work here presented, is that the treatment of chronic syphilitic diseases of the central nervous system is not the administration of two or four intravenous injections of salvarsan, or the exhibition of a certain amount of mercury and iodids. Each case presents a slightly different problem, and must be considered individually. Some respond much more rapidly than others, but the object in all should be to obtain a persistently normal cerebrospinal fluid. Of course, we cannot state with certainty that a normal cerebrospinal fluid assures that there will be no further progress of the degenerative process. All we can say is that as long as the cerebrospinal fluid gives evidence of a specific pathological process in the central nervous system, and we have specific therapeutic measures which will remove that evidence, these measures should be applied. Only when the fluid has become normal are we justified in discontinuing the treatment, and observations of the fluid should be continued so that at the first evidence of relapse treatment may be resumed.

The method of intraspinous injection is not presented as a substitute for any of the accepted forms of treatment, but as an aid in attacking these severe infections. We feel that there is definite evidence that this form of treatment has a curative action on the syphilitic process, and that, therefore, its combination with intensive intravenous treatment is indicated where specially intensive treatment is required, as in rapidly advancing tabes or paresis, or where the disease has resisted other forms of treatment.

THE VALUE OF EDESTIN AND PEPTONE IN THE DIAGNOSIS OF CANCER OF THE STOMACH *

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Neubauer and Fischer,[1] in 1909, proposed the use of glycyltryptophan as a reagent for the estimation of peptolytic activity and applied it to the diagnosis of carcinoma of the stomach. The test consisted in mixing glycyltryptophan with a portion of the filtered gastric juice obtained after the usual test breakfast, incubating for twenty-four hours, and then testing with bromin vapor for the rose-violet color of free tryptophan.

Their conclusions were as follows:

1. There exists in carcinomatous stomach contents a ferment which, contrary to pepsin, will split glycyltryptophan.

2. This ferment is destroyed by an acidity of .36 per cent. HCl.

3. The presence of this ferment may be of diagnostic value.

Their report was accepted with widely divergent criticism. Lyle and Kober,[2] Pechstein,[3] Oppenheimer[4] and others, considered it of value. Ley,[5] Ehrenberg,[6] Sanford and Rosenbloom,[7] etc., believed it valueless. Several authors described modifications of the original method. Weinstein[8] tested stomach contents directly for free tryptophan; Kuttner and Pulvermacher[9] used silk peptone as a less expensive reagent, estimating peptolytic action by the precipitation of tyrosin under the microscope; Jacque and Woodyatt[10] and Schryver and Singer[11] proposed a quantitative test, applying the formol titration of Sorensen-Ronchese to a mixture of gastric juice and Witte peptone. None of these modifications, with the exception of the last-named, have yielded more uniform results than the original method. In the procedure about to be described we have used the peptone quantitative method and have added the use of edestin

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1. Neubauer and Fischer: Deutsch. Arch. f. klin. Med., 1909, xcvii, 499.
2. Lyle and Kober: New York Med. Jour., 1910, xci, 1151.
3. Pechstein: Berl. klin. Wchnschr., 1911, xlviii, 375.
4. Oppenheimer: Deutsch. Arch. f. klin. Med., 1910-11, ci, 293.
5. Ley: Berl. klin. Wchnschr., 1911, xlviii, 119.
6. Ehrenberg: Berl. klin. Wchnschr., 1911, xlviii, 704.
7. Sanford and Rosenbloom: THE ARCHIVES OF INT. MED., 1912, ix, 445.
8. Weinstein: Jour. Am. Med. Assn., 1911, lvii, 1420.
9. Kuttner and Pulvermacher: Berl. klin. Wchnschr., 1910, xlvii, 2057.
10. Jacque and Woodyatt: THE ARCHIVES INT. MED., 1912, x, 560.
11. Schryver and Singer: Quart. Jour. Med., 1912, vi, 71.

as a means of controlling the most frequent source of error — proteolytic cleavage.

One of the most frequent criticisms of the biochemical tests for cancer of the stomach is the fact that the gastric contents of normal individuals, as well as of non-cancerous patients, under certain conditions, split glycyltryptophan and other polypeptids. Although several authors have defined the sources of error in these tests and have suggested various modifications to control them, no one, so far as we are aware, has made use of a second substrat to control and rule out the cleavage due to non-cancerous ferments. In view of these considerations, we propose, therefore, to discuss, first, the probable sources of error; second, to submit a method for the control of the most frequent error — proteolytic cleavage — third, to report a series of cases in which this method has been used.

1. *The sources of error in the ability of gastric contents to split polypeptids in the absence of malignant disease.*

Emerson,[12] following the suggestion of Friederich Müller, showed that the unusually deep cleavage of proteins by gastric contents of cancer patients was due to the activity of a ferment derived from the cancer cells. This ferment Abderhalden[13] believed belonged to the general group of ereptases, ferments capable of splitting polypeptids and peptones, but incapable of attacking native protein. While minor differences have been found to exist between cancer ereptase and the universal ereptases of normal tissues, no specificity has been noted so far as the cleavage of polypeptids and peptone is concerned. It has been shown further by one of us,[14] that quantitatively cancer cells of the epithelioid type contain less ereptase than normal kidney and liver cells (dog), but more than normal spleen and blood serum; while cancers of the connective tissue type contain least of all. It should be emphasized, therefore, that these tests based on peptolytic cleavage are specific only insofar as the ereptase of any given gastric contents can be shown to be derived from the cancer tissue itself. To do so, we must rule out ereptase from non-cancerous sources, as follows:

(a) The ereptase (erepsin) of regurgitated duodenal contents (succus entericus).

(b) Serum ereptase from hemorrhage into the stomach and from transudation of ereptase-containing fluids into the stomach.

(c) Tissue ereptase from breaking down of cells of the gastric mucosa.

(d) Ereptase of swallowed saliva.

In addition, protease, capable of peptolytic cleavage, as well as proteolytic cleavage, must be identified and controlled, if present:

(a) Trypsin of regurgitated pancreatic juice.

(b) Proteases of bacteria and leukocytes — mostly from swallowed saliva.

12. Emerson: Deutsch. Arch. f. klin. Med., 1902, lxxii, 415.

13. Abderhalden: Ztschr. f. Krebsforsch., 1910, ix, 226.

14. Hamburger: Jour. Am. Med. Assn., 1912, lix, 847.

From a general survey of these several sources of error, we considered, for reasons to be discussed later, that the second group, the proteases of pancreatic juice (trypsin) and saliva (leukocytes and bacteria), were most frequently active. We believed, therefore, that if this proteolytic cleavage could be controlled, we should materially increase the reliability of the methods in question. Our results (described in Section 3) would lend support to this view.

The presence of pancreatic trypsin as a disturbing factor in the glycyltryptophan test was recognized by Neubauer and Fischer in their original publication and was controlled by discarding bile-stained gastric contents.

Kuttner and Pulvermacher, Lyle and Kober, Öhrl and Schittenhelm[15] and others, contended subsequently that the bile test was inadequate to exclude trypsin. Boldyreff has shown recently that a yellow, clear fluid —*free from bile,* rich in carbonates (pancreatic juice) and containing pancreatic ferments, flowed from the gastrostomy tube of a Pawlow dog, following the withdrawal of a weak hydrochloric acid solution, introduced for the purpose of stimulating the secretion of pancreatic juice. On the basis of our own work we agree with those holding that a negative bile test does not of itself exclude trypsin.

Warfield[16] and Koehlker[17] first called attention to the fact that saliva under certain conditions is capable of hydrolyzing gycyltryptophan and other di- and tripeptids, believing such action to be due to a hitherto undescribed salivary ferment. Jacque and Woodyatt and Smithies,[18] experimenting with saliva after passage through a Berkefeld filter and centrifuging, conclude that the peptolytic action is due, at least in large part, to bacteria. From certain experiments of our own, we have found in various salivas evidences of proteolytic (as well as peptolytic) activity, due probably to the contained leukocytic ferments (leukoprotease). This leukoprotease we believe to be responsible for much of the salivary peptolytic action. We would further emphasize that the method about to be described for the control of pancreatic trypsin serves equally well for salivary leukoprotease, thereby dismissing, for purposes of clinical diagnosis, the problem of the origin of the proteolysis.

2. *The use of edestin and peptone in differentiating and estimating proteolytic and peptolytic cleavage.*

The use of the vegetable globulin edestin was suggested to us by Dr. Jobling as a true native protein subject to cleavage by proteolytic fer-

15. Öhrl and Schittenhelm: Zentralbl. f. d. gesamt. Physiol. u. Path. d. Stoffwechs., 1912, xi, 881.

16. Warfield: Bull. Johns Hopkins Hosp., 1911, p. 150.

17. Koehlker: Ztschr. f. physiol. Chem., 1911, No. 30.

18. Smithies: THE ARCHIVES INT. MED., 1912, x, 521.

ments only, thereby serving as a substrat for differentiating cleavage due to peptolytic enzymes. Casein, which was used to some extent in our earlier experiments, was later discarded in view of its slight digestion by erepsin (Cohnheim). Fischer and Abderhalden[19] and Abderhalden and Gigon[20] have previously used edestin in estimating the activity of pancreatic juice and ferments, while Fuld and Levison[21] have used it for quantitative determinations of pepsin. These several procedures are based on the fact that edestin is soluble in both acids and alkalies, but is insoluble in neutral solutions, particularly in the presence of concentrated salt solution. (Osborne.)

METHOD

(*a*) *Control and Estimation of Proteolytic Cleavage* (*Proteolysis*).—Crystallyzed edestin, prepared by macerating hemp seeds in 10 per cent. NaCl, then dialyzed and precipitated with 95 per cent. alcohol, is made up as a 0.1 per cent. solution in 0.1 per cent. Na_2Co_3. This stock solution in the refrigerator remains unchanged for several days to a week.

The filtered (through ordinary moistened filter paper) gastric contents, obtained as soon as possible after the usual Ewald breakfast, are neutralized with a normal Na_2CO_3 solution (2 drops of a 1 per cent. alcoholic phenolphthalein solution serving as indicator) and brought to an alkalinity equal to N/100 Na_2CO_3, for the purpose of inactivating pepsin while activating trypsin and other alkaline and neutral acting proteases.

Two c.c. of the edestin solution is placed in each of four ordinary test-tubes, and the neutralized gastric filtrate added in decreasing amounts of 2 c.c., 1 c.c., 0.5 c.c., respectively, to the first three test-tubes; the fourth tube, containing edestin and carbonate alone, serving as control. All four tubes are made up to an equivalent volume with a 1 per cent. Na_2CO_3 solution. The tubes are then placed in the incubator at 37 C., and removed at the end of four hours. Proteolysis is estimated by the precipitation of unhydrolyzed edestin by the drop by drop addition of 5 per cent. acetic acid; complete absence of turbidity on neutralization indicating complete digestion; slight turbidity, partial digestion; maximum turbidity (as compared with the control tube), no digestion. The degree of proteolysis is indicated in the protocols as follows: Complete digestion in all tubes (except control) (no precipitate on neutralization), + + +; partial digestion (no precipitate in Tubes 1 and 2, with turbidity in Tube 3), + +; minimal digestion (no precipitate in Tube 1, with turbidity in 2 and 3), +; no digestion (turbidity in all tubes), 0.

19. Fischer and Abderhalden: Ztschr. physiol. Chem., 1905, xlvi, 52.
20. Abderhalden and Gigon: Ztschr. physiol. Chem., 1907, liii, 119.
21. Fuld and Levinson: Biochem. Ztschr., 1907, vi, 473.

Care is necessary that only the minimum of acid necessary to neutralize be added, as excess of acid causes a re-solution of edestin. It is well to standardize the number of drops of acid necessary for neutralization in the control tube with the aid of a drop of phenolphthalein.

(*b*) *Estimation of Peptolytic Cleavage* (*Peptolysis*).—Ten c.c. of the gastric filtrate are added to 20 c.c. of a 2 per cent. Witte's peptone solution and 10 c.c. of the mixture removed as control and titrated for free amino groups by the Sorensen-Ronchese formol method, as applied by Jacque and Woodyatt. The remaining 20 c.c. are placed in an Erlenmeyer flask under toluol at 37 C. for twenty-four hours; at the end of that period, 10 c.c. are again removed and titrated, the difference between the first and the second titration expressing the degrees of peptolysis in terms of c.c. N/10 NaOH per 100 c.c. of the mixture.

3. *Results of the application of the edestin peptone method in a series of cases.*

This report comprises the results obtained from thirty-seven cases, divided as follows:

	Cases
(a) Cancer of the stomach	10
(b) Chronic ulcer of the stomach	5
(c) Chronic inflammation of gall-bladder and pancreas	6
(d) Old gastro-enterostomy cases	4
(e) Control cases	12
Total	37

Only those cases are included in which the diagnosis was controlled by the operative or autopsy findings, or in which the clinical picture was so definite as practically to exclude errors in its interpretation.

Our conclusions may be summarized as follows, and we would emphasize that they are submited as preliminary only, as they are drawn from too small a number of cases to be considered as final.

(*a*) *Cancer of the Stomach* (Table 1).—Moderately advanced and advanced cancer of the stomach, involving the pyloric portion, associated with low total acidity and low or absent free HCl produces greatest peptone cleavage (peptolysis 8.2; proteolysis trace).

Early cancer, situated on the lesser curvature between pylorus and cardia, possibly developing on an ulcer basis, with high total acidity and low or absent free HCl, gives least peptone cleavage (peptolysis 2.0; proteolysis 0).

Microscopic and chemical tests for bile are not sufficient to rule out tryptic proteolysis in all cases. Edestin is a reliable means of controlling proteolytic cleavage from pancreatic juice and saliva.

TABLE 1.—Cancer Cases. (Ten Cases.)

Case No.	Ewald						Peptone			Edestin	Diagnosis
	Amt. c.c.	Free HCl	Total Acid	Lactic	Bile	Blood	Control	Twenty-four Hour	Inc.		
1	110	0	9	0	0	0	10	18	8	*	Refused operation. Clinical diagnosis carcinoma; hard, palpable tumor mass.
2	110	0	8	0	0	0	10	25	15	*	Operation. Carcinoma, involving pylorus and one-third greater curvature. Pylorus partially stenosed.
3	70	0	20	++	0	0	13	19	6	*	Operation. Carcinoma of pylorus and greater and lesser curvature. Pylorus completely stenosed by foreign body.
4	130	0	42	0	0	0	10	12	2	*	Operation. Interosteal carcinoma with perforation; lesser curvature. Probable cancer on old ulcer.
5	150	0	12	++	0	†	11	22	11	++	Refused operation. Clinical diagnosis, carcinoma.
	200	0	6	+	++	0	11	26	15	++	
6	?	0	12	0	0	0	14	16	2	0	Operation. Carcinoma anterior wall near lesser curvature.
7	275	12	40	0	0	++	..	..	..	....	Operation. Carcinoma greater part lesser curvature and posterior wall; probable development of carcinoma from preceding gastric ulcer.
	200	8	37	0	0	+	13	13	0	0	
8	‡	15	85	0	0	++	15	15	0	0	Operation. Carcinoma anterior wall of stomach developing from gastric ulcer. Mistaken diag. No evidence of malignancy (?)
9	120	0	6	+	0	0	7	15	8	0	Operation refused. Clinical diagnosis, carcinoma.
10	?	0	32	+	0	0	15	30	15	*	Operation for stenosing carcinoma of pylorus and posterior wall and lesser curvature.

* Not made. † Trace. ‡ Vomitus.

TABLE 2.—Ulcer Cases. (Five Cases)

Case No.	Ewald						Peptone			Edestin	Diagnosis
	Amt. c.c.	Free HCl	Total Acid	Lactic	Bile	Blood	Control	Twenty-four Hour	Inc.		
11	140	62	79	0	0	0	9	9	0	0	Duodenal ulcer.
12	130	0	8	+	†	0	18	18	0	++	Operation; chronic ulcer at duodenal pylorus juncture. No evidence of malignancy.
13	210	26	42	0	0	0	11	12	1	0	Chronic pyloric ulcer.
14	100	35	68	0	0	0	15	16	1	*	Chronic duodenal ulcer.
15	80	44	100	0	0	0	15	16.5	1.5	*	Chronic gastric ulcer.

* Not made.

Lactic acid is not a marked inhibiting agent of peptolysis; blood, high total acidity and moderate free HCl (6 to 20) cause greatest (to complete) inhibition.

(*b*) *Chronic Ulcer of the Stomach and Duodenum* (Table 2).—Chronic ulcer associated with high free HCl and high total acidity produces practically no proteolytic or peptolytic cleavage (peptolysis 0.5; proteolysis 0).

In one instance (Case 12) chronic ulcer with absent free HCl and a total acidity of 8 gave *no* peptolytic cleavage, but positive proteolytic digestion.[22]

TABLE 3.—GALL-BLADDER AND PANCREAS CASES. (SIX CASES)

Case No.	Ewald						Peptone			Edestin	Diagnosis
	Amt. c.c.	Free HCl	Total Acid	Lactic	Bile	Blood	Control	Twenty-four Hour	Inc.		
16	25	0	25	0	+	0	11	14	3	*	Refused operation. Chronic cholecystitis and cholelithiasis.
17	90	0	11	0	0	0	11	24	13	++	Refused operation. Alcoholic gastritis; chronic cholecystitis.
18	?	0	10	+?	0	0	9	20	11	+++	Operation. Chronic cholecystitis and pancreatitis.
19	50	0	34	0	0	0	9	15	6	++	Refused operation. Cholecystitis and cholelithiasis.
20	60	0	19	0	0	0	14	30	16	*	Refused operation. Chronic cholecystitis and gastritis.
21	50	0	10	+?	0	0	11	24	13	+	Operation. Chronic cholecystitis with cholelithiasis; pancreatitis.

* Not made.

(*c*) *Chronic Inflammations of Gall-Bladder and Pancreas* (Table 3).—Chronic cholecystitis and pancreatitis associated with absent free HCl and low or moderate total acidity, with negative tests for bile, cause both marked proteolysis and peptolysis (peptolysis 11 to 14.5; proteolysis + to + + +).

In such cases edestin is an effective control of proteolytic cleavage, in particular where the clinical and laboratory findings point strongly to cancer. In our thirty-seven cases, six showing marked peptolysis with negative bile tests were so controlled by edestin, thereby reducing a possible 16 per cent. error to zero.

22. A larger series may show this to possess differentiating value between ulcer and early cancer, viz: absent peptolysis with positive proteolysis (with low acidities) speaking for ulcer; moderate peptolysis with moderate or absent proteolysis speaking for cancer.

(*d*) *Old Gastro-Enterostomy Cases* (Table 4).—In old gastro-enterostomy cases (for benign pyloric obstruction) with positive bile tests, proteolysis and peptolysis are both marked (peptolysis, 16.6; proteolysis, +++).

It is of interest to note the parallel tests of bile and edestin as controls of proteolytic cleavage in this series, as compared with the negative bile and positive edestin tests of Table 3. It would appear that either bile experiences less difficulty in gaining entrance to the stomach through the gastro-enterostomy opening, or, and more likely, the stimulus to biliary secretion is increased by certain physiologic changes incident to the deflection of the chyme stream.

(*e*) *Control Cases* (Table 5).—Series of control (normal) cases show uniform absence of proteolysis and peptolysis in presence of free HCl, and in some cases positive proteolysis with negative bile tests (peptolysis, 0 to 4; proteolysis, 0 to +).

The degree of peptolysis and proteolysis in these several groups of cases may be tabulated as follows:

	Peptolysis Average	Proteolysis Average
Cancer (10 Cases)—		
7 Pyloric carcinomas	8.2	trace
2 Interosteal carcinomas	2.0	0
1 Lesser curv. and post. wall	0.0	0
Ulcer (5 Cases)—		
3 Chronic pyloric ulcers	0.5	+
2 Chronic duodenal ulcers	0.5	0
Gall-Bladder and Pancreas (6 Cases)—		
2 Chronic cholecystitis with cholelithiasis	4.5	+
2 Chronic cholecystitis with gastritis	14.5	++
1 Chronic cholecystitis with pancreatitis	11.0	+++
1 Chronic cholelithiasis with pancreatitis	13.0	+
Gastro-Enterostomy (4 Cases)—		
3 Old incompetent gastro-enterostomies for gastric ulcer with pyloric stenosis	16.6	+++
1 Old gastro-enterostomy or duodenal ulcer	0.	++
Control Cases (12 Cases)		
5 Neurasthenia, sec. anemia, gastroptosis, etc.	0.	0
7 Pernicious anemia, Basedow, acute nephritis, etc.	4.	+

These results may be taken to indicate that in cases of doubtful diagnosis in which cancer is being considered, a relatively high peptolysis with low proteolysis speaks for carcinoma; high peptolysis and high proteolysis against carcinoma.

TABLE 4.—Gastro-Enterostomy Cases. (Four Cases)

Case No.	Ewald						Peptone			Edestin	Diagnosis
	Amt. c.c.	Free HCl	Total Acid	Lactic	Bile	Blood	Control	Twenty-four Hour	Inc.		
22	180	29	56	0	+++	0	12	12	0	++	Old, incompetent gastro-enterostomy for chronic duodenal ulcer.
23	120	0	4	0	++	0	13	34	21	+++	Old, incompetent ("vicious circle") gastro-enterostomy for gastric ulcer.
24	75	0	16	0	++	0	11	35	24	++	Old, post gastro-enterostomy for pyloric ulcer. Sarcoma of intestine.
25	80	37	62	0	++	0	7	12	5	++	Old (eighteen months), gastro-jejunostomy for supposed pyloric ulcer. Operation disclosed a carcinomatous ulcer in upper jejunum, opposite gastro-jejunal opening.

* Not made.

TABLE 5.—Control Cases. (Twelve Cases)

Case No.	Ewald						Peptone			Edestin	Diagnosis
	Amt. c.c.	Free HCl	Total Acid	Lactic	Bile	Blood	Control	Twenty-four Hour	Inc.		
26	...	..	..	..	....	..	6	10	4	*	
27	50	12	38	X	0	0	9	18	9	+	Pernicious anemia.
28	100	20	45	0	0	0	16	16	0	0	Severe secondary anemia from unknown cause. [Pernicious anemia (?).]
29	130	9	25	0	0	0	10	10	0	0	Gastroptosis; secondary anemia; chronic interstitial nephritis.
30	...	13	43	..	....	..	..	..	..	....	Membranous colitis.
31	40	18	36	0	0	0	11	11	0	*	Thrombo-angiitis obliterans. (Splanchnic vessels.)
32	15	0	15	0	0	0	13	16	3	+	Membranous colitis.
33	110	25	66	..	....	..	15.5	16	1.5	*	Neurasthenia.
34	...	?	..	..	....	..	12	15	3	*	Neurasthenia.
35	97	0	13	0	0	0	12	19	7	++	Refused operation. Carcinoma descending colon; enteroptosis.
36	...	..	?	..	....	..	11	13	2	+	Exophthalmic goiter.
37	160	..	?	..	....	..	11	14	3	*	Acute nephritis.
	...	..	?	..	....	..	13	14	1	*	Gastric neurosis.

* Not made.

SUMMARY

1. Edestin is a valuable aid in controlling the proteolytic cleavage of stomach contents.

2. The proteolytic cleavage of stomach contents is due in most instances to regurgitated trypsin, although leukocytes and bacteria probably play some rôle.

3. By the use of edestin with peptone it is possible to materially reduce the errors in non-cancerous and normal cases due to trypsin, leukocytes and bacteria.

4. The edestin-peptone method possesses distinct value in the diagnosis of cancer of the stomach and is of considerable service in the differential diagnosis between benign and malignant anacidity.

5. High peptolysis with low proteolysis speaks for carcinoma; high peptolysis with high proteolysis against carcinoma.

6. The edestin-peptone method, as in other laboratory tests, is of practical value only when taken in conjunction with the usual clinical and laboratory findings.

The cases in this report were taken for the most part from the regular medical and surgical services of Michael Reese Hospital. Our thanks are given to the attending men and interns on the services for their cooperation and courtesy in placing these cases at our disposal. We are likewise indebted to Dr. Julia Crotty, of the Cook County Hospital, for two cases from the medical wards of that hospital. Finally, we would express our appreciation to Dr. Jobling for helpful criticisms and suggestions.

BOOK REVIEW

TUBERCULIN IN DIAGNOSIS AND TREATMENT. By Louis Hamman and Samuel Wolman. D. Appleton & Co., New York and London, 1912.

During the last ten years the use of tuberculin in diagnosis and treatment has received wide attention, but so numerous are the preparations of tuberculin now available, so varied their methods of application, and so diversified the views as to the choice of preparation, the value of tuberculin in diagnosis and its place as a curative measure, that it requires much time and accurate knowledge of the voluminous literature to acquaint oneself with the necessary details and current opinions. All this accumulated and valuable knowledge Hamman and Wolman have sifted and arranged, together with their own extensive experience, into a very interesting and exceedingly useful book. The subject is treated from its broadest and most rational aspect, and presented in a thorough and impartial manner.

The book of some three hundred and fifty pages is divided into three parts: the first deals with "The scientific principles underlying the diagnostic and therapeutic use of tuberculin"; the second with "The use of tuberculin in diagnosis," and the third with "The use of tuberculin in treatment." The first of these parts or chapters embodies an excellent résumé of the experimental basis for the practical application of tuberculin, and the theoretical problems connected therewith, and includes an interesting account of the gradual development of our knowledge concerning the tuberculin reaction and its relation to anaphylaxis. In the second part the various methods of applying tuberculin for diagnostic purposes are carefully described and the practical worth of these different methods analyzed and compared. Hamman and Wolman speak quite favorably of the conjunctival reaction, which in their hands has not been attended with any alarming results and which they think if used properly is harmless. They consider it the least sensitive test for the determination of the presence of a tuberculous focus in the body, but the most valuable positive test for determining the presence of an active tuberculous infection. The cutaneous and intracutaneous tests are so delicate that they can be used only to exclude the presence of tuberculosis. In the last part there is a thorough discussion of tuberculin as a therapeutic measure. The authors believe that it has value analogous to fresh air, rest and diet, and since it is an adjuvant if used properly, its worth should not be neglected. There follow descriptions of the different tuberculins now in use, their method of application in tuberculosis of different organs and the dangers dependent on their misuse.

The book represents a most careful and critical survey of the whole subject which is presented in an interesting and really illuminating manner.